Introduction of new MS instrument

Proteomics: Karel Harant
               Pavel Talacko
               Veronika Ševců

Metabolomics: Olga Součková
                 Petr Žáček

Equipment

GC/GC/MS
Leco Pegasus

Triple Quadrupole
Quantiva

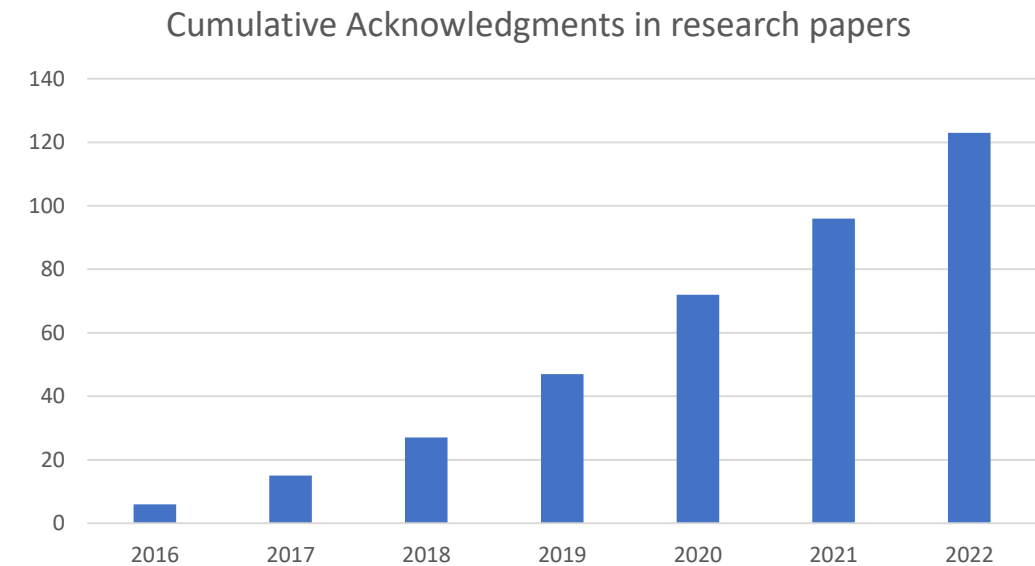Fusion   Ascend

Tribride Orbitrap

Services

- Proteomics
  - Sample preparation
  - Global proteome profiling
  - MS analysis of Immunoprecipitation experiments
  - Phoshoproteomics – global phosphoproteome
  - **Isobaric labeling quantification**
  - Target peptide measurement
  - Absolute quantification of peptides
  - Basic statistics and bioinformatics analysis

- Metabolomics
  - Tailor-made analyses of selected metabolites
  - Untargeted and targeted analyses
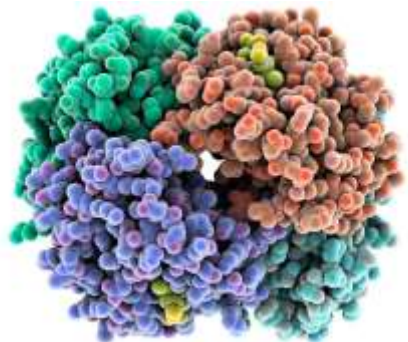 - Absolute quantification of selected metabolites

Please contact us before the start of the experiment

Contemporary state and Brief history

- Established in 2014 - Orbitrap Fusion

- 2015 - triple quadrupole Quantiva and GC/GC/Pegasus – LECO

- 2022 – Spetronaut software and DIA method available for users

- **2023 – Orbitrap Ascend – dedicated to EXCELES NIVB**

- 125 acknowledgments in research papers

- 4 MS instruments

- 5 staff scientist
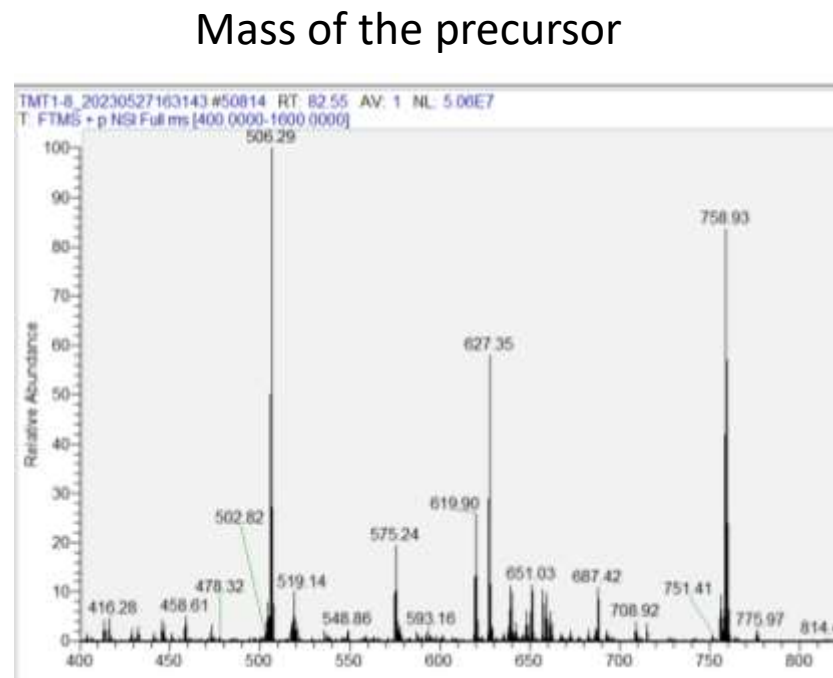
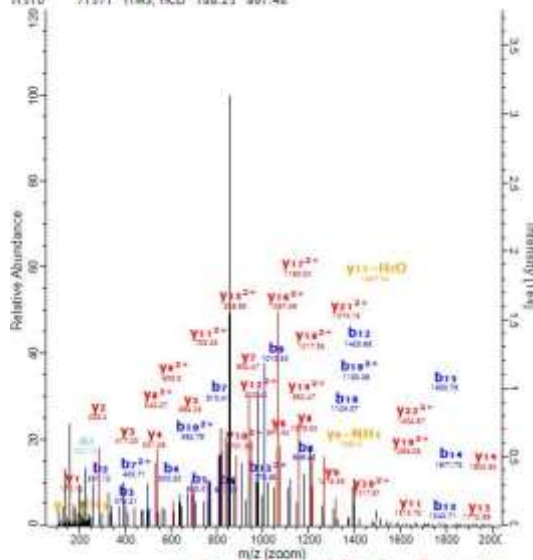- 280 user access per year

- 3000 samples per year

Cumulative Acknowledgments in research papers

Proteomics workflow

Trypsin digestion

Peptides

LC/MS

Mass of the precursor
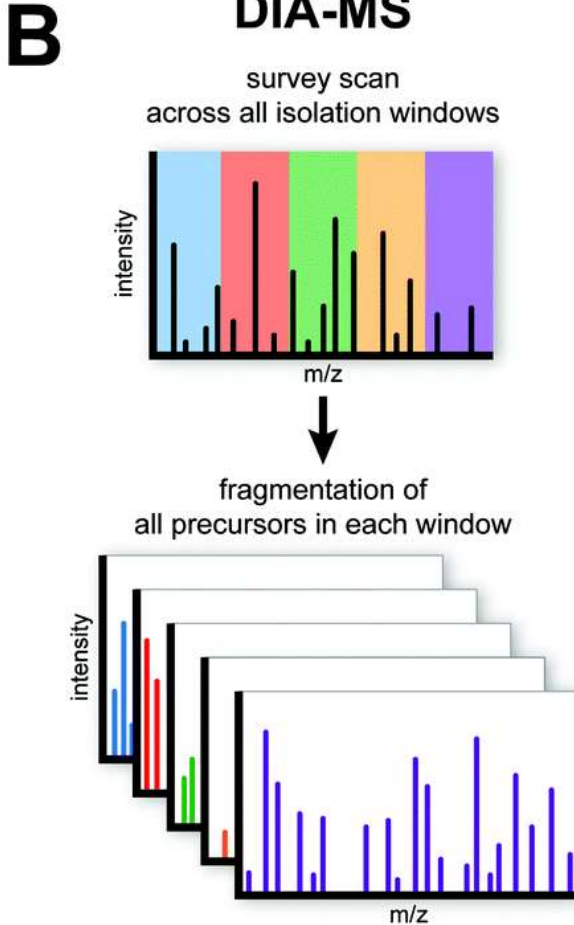
Precursor fragmentation

Fragmentation spectrum – peptide identification

Data-dependent and data independent

- Isolate one precursor for each fragmentation
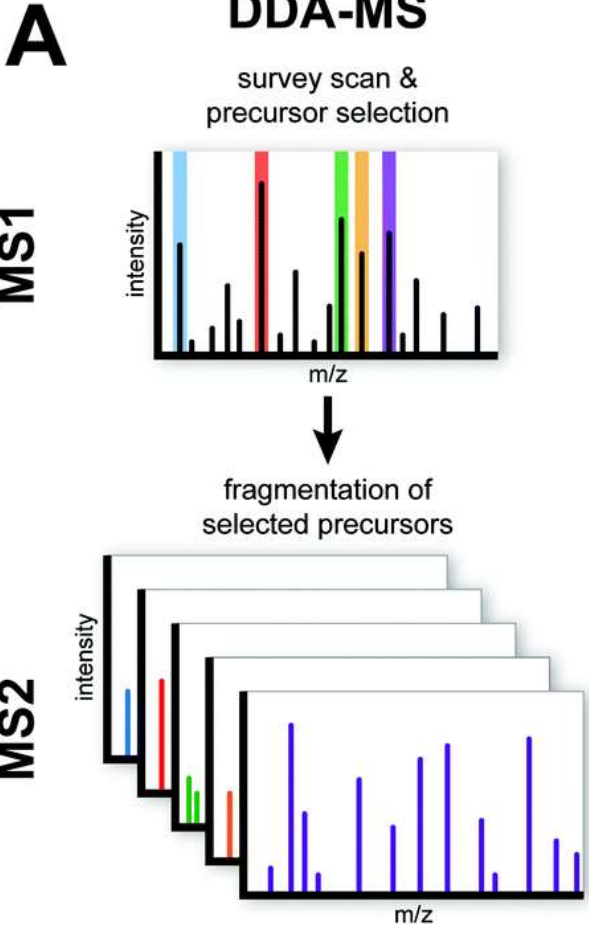
- Idealy no co-isolation

- Cyclic Isolation of wide mass windows

- Quantification and identification from MS^2

- Intentional consolation

- Deconvolution of mixed s processing

- Higher number of Ids and missing values

essing of data

values

an

Orbitrap Ascend

- Fourth iteration of tribrid instruments

- Combination of three types of mass analyzers

- Quadrupole for fast precursor selection

- Orbitrap for precise and high-resolution measurement

- Ion Trap for higher orders of fragmentation.
  And for fast and sensitive measurement

- For proteomics is the most important speed of data acquisition - parallelization of data acquisition

- Allow usage of different combinations of mass analyzers to reach the best results

- Allow MS^3 fragmentation

- Allow online peptide search and choose precursors for MS^3 fragmentation according to the search results
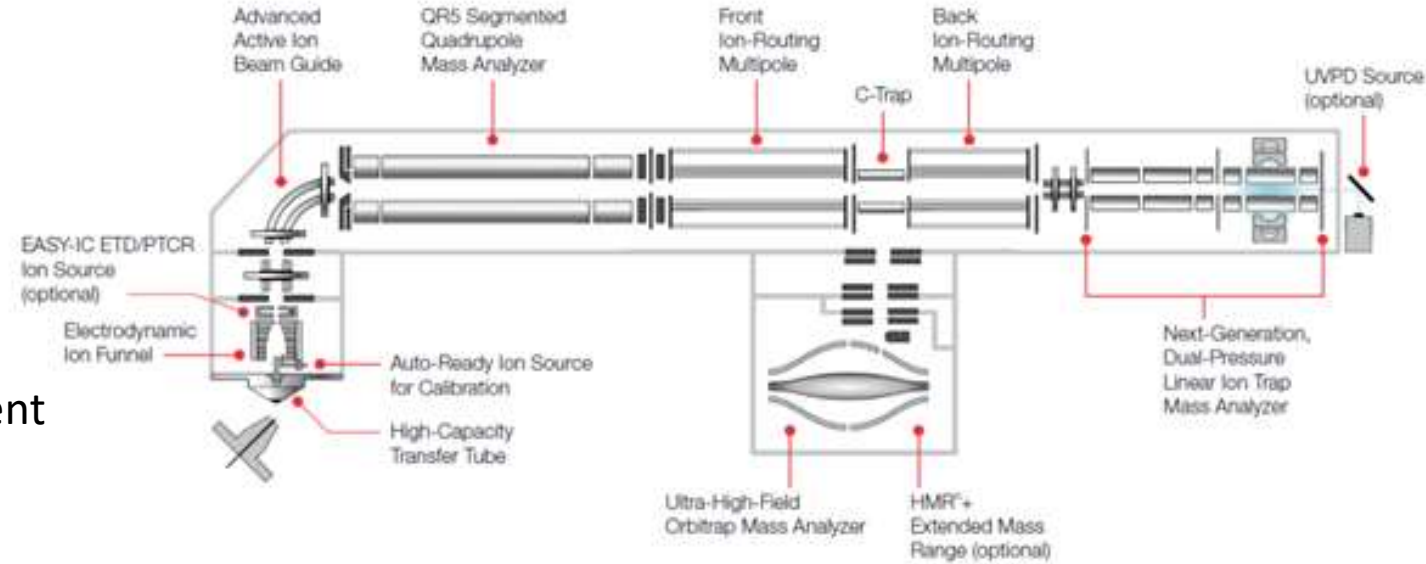
Protein quantification possibilities

- **Labell free**

- Plain comparison of peak areas

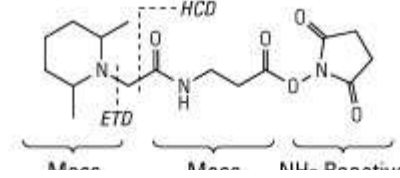- More missing values with DDA

- Less deep proteome coverage

- **Multiplexed labeled samples**

- High data completness

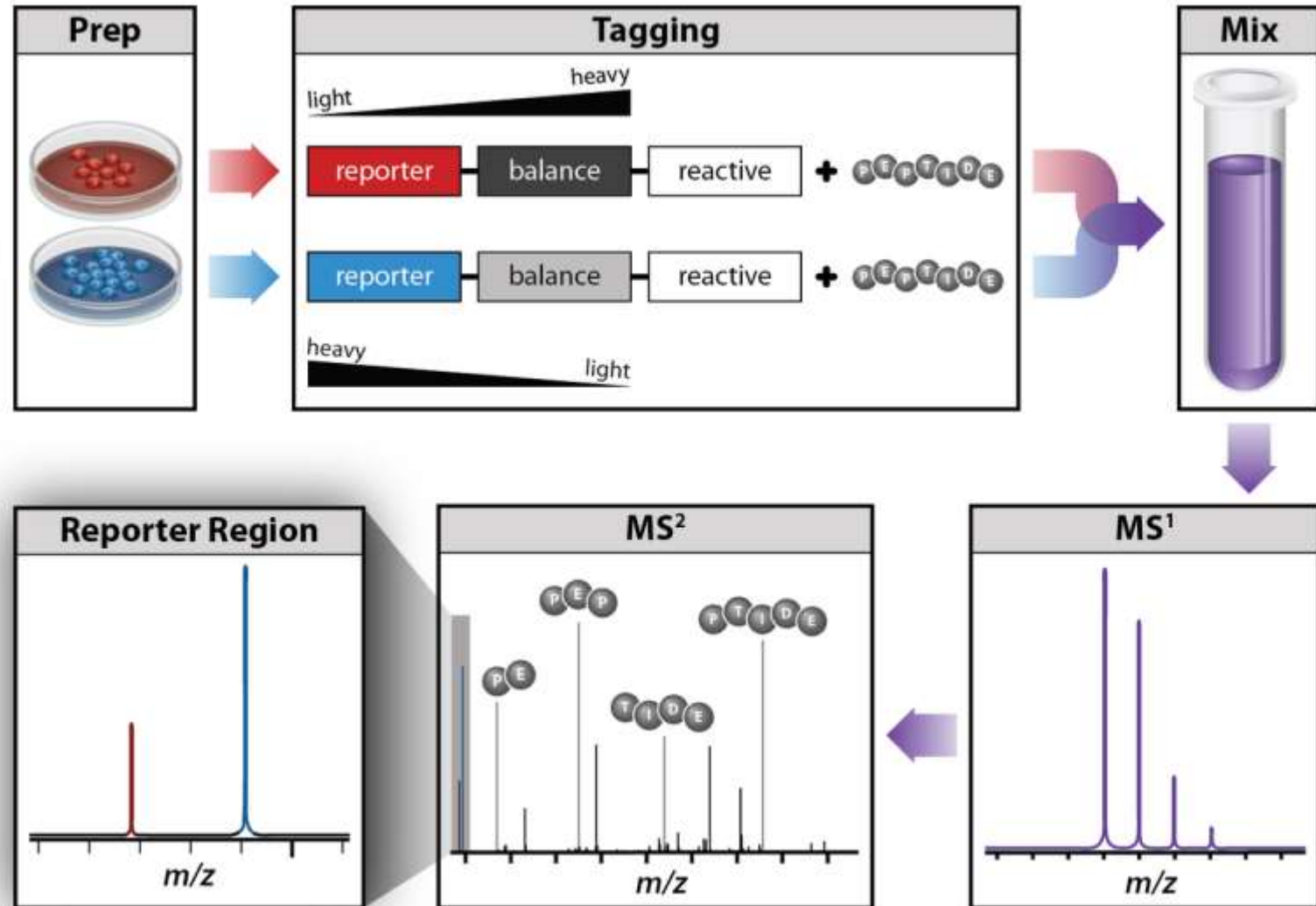- Reduce sample preparatinon artefacts

- Reduce chromatography artefacts
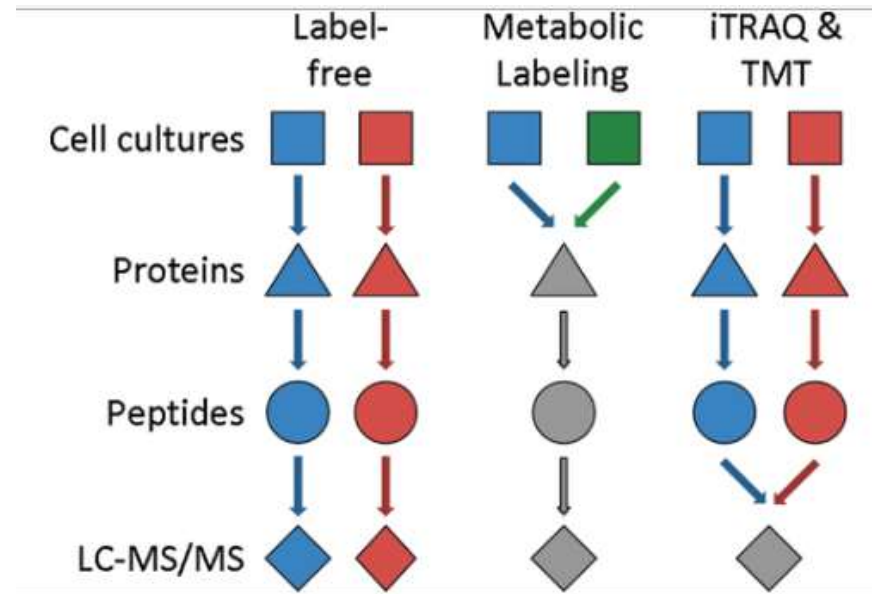
Isobaric labeling – TMT, ITRAQ
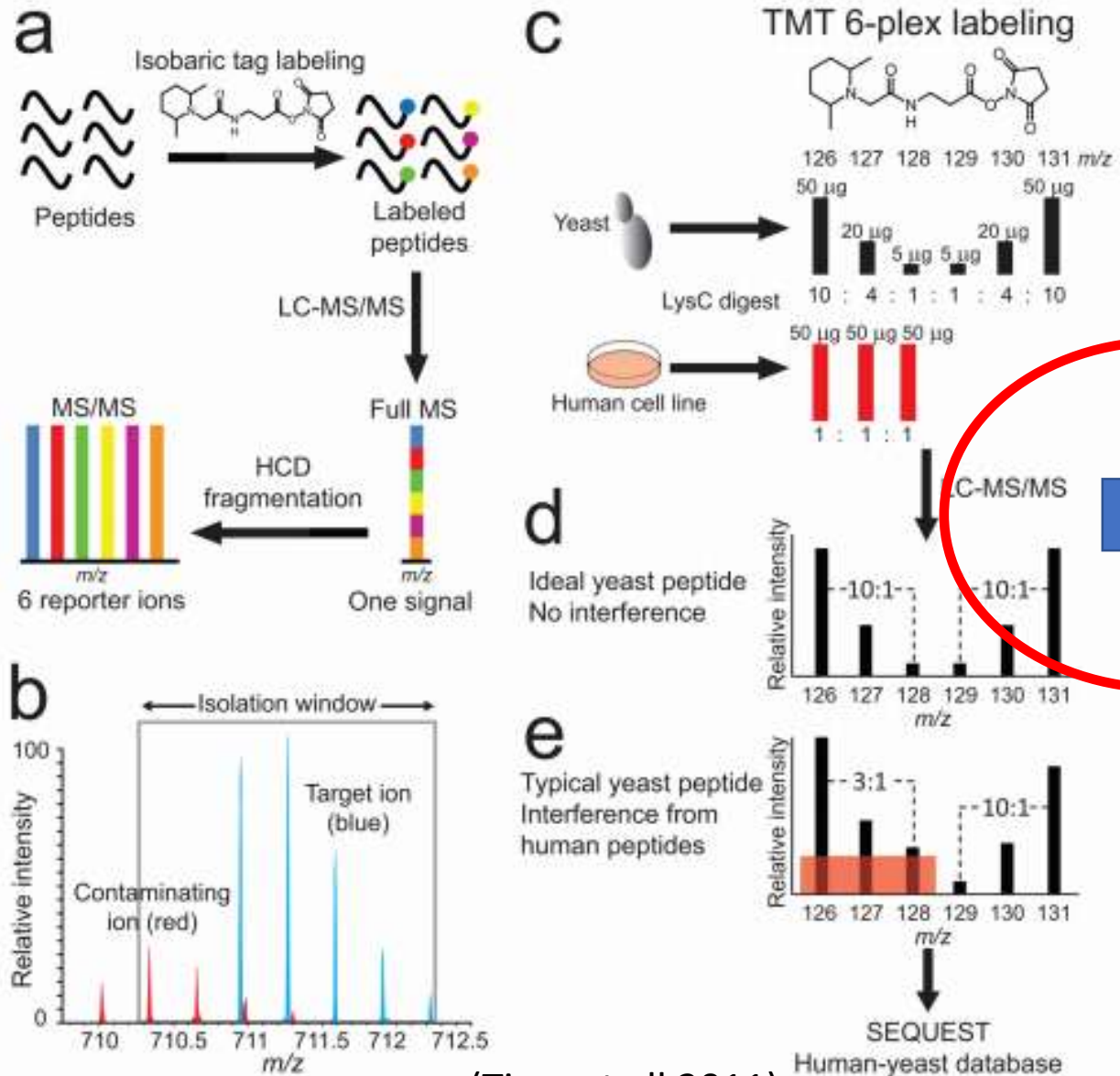

A. TMT Reagent Generic Chemical Structure
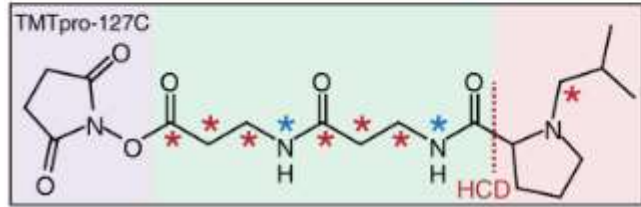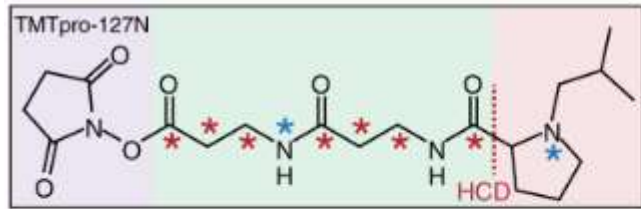
- Multiplexing approach
- 18 channels available today
- Reduce instrument time
- Data completeness
- Slower data acquisition
- Necessity of fractionation

Coisolation – ratio compression

MS³ quantification

Time consuming step,
With real-time search you can fragment just
Precursors with valid peptide identification
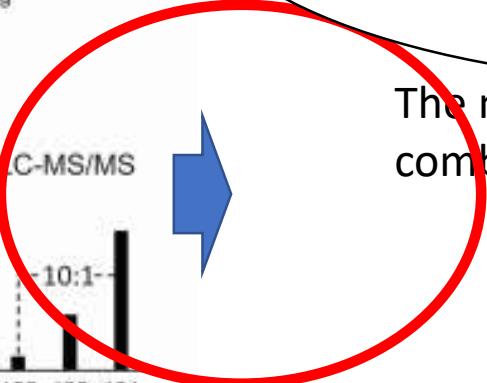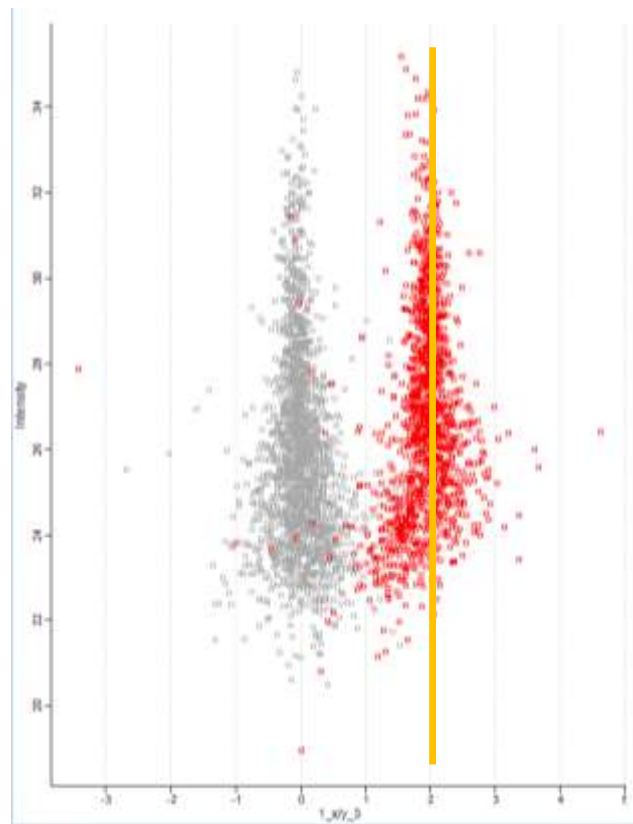
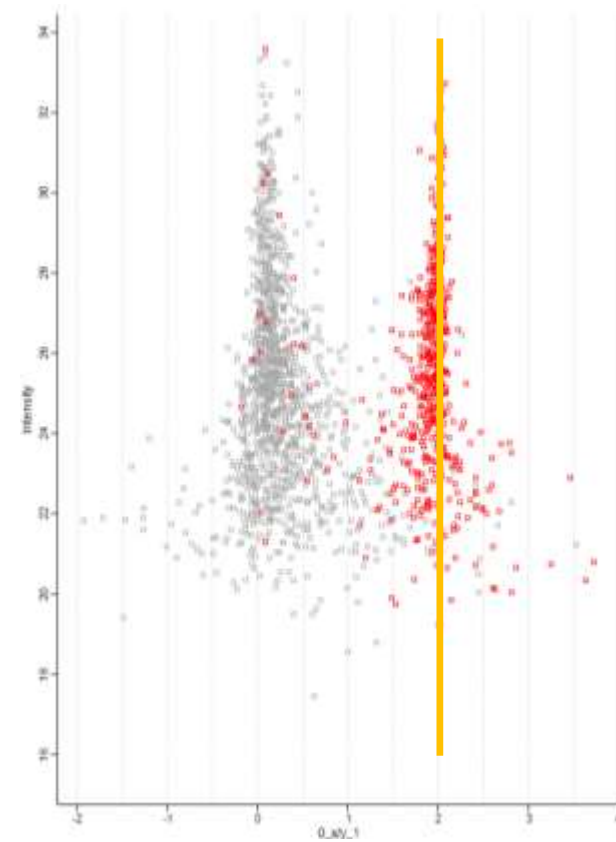The necessity of ion trap and orbitrap
combination
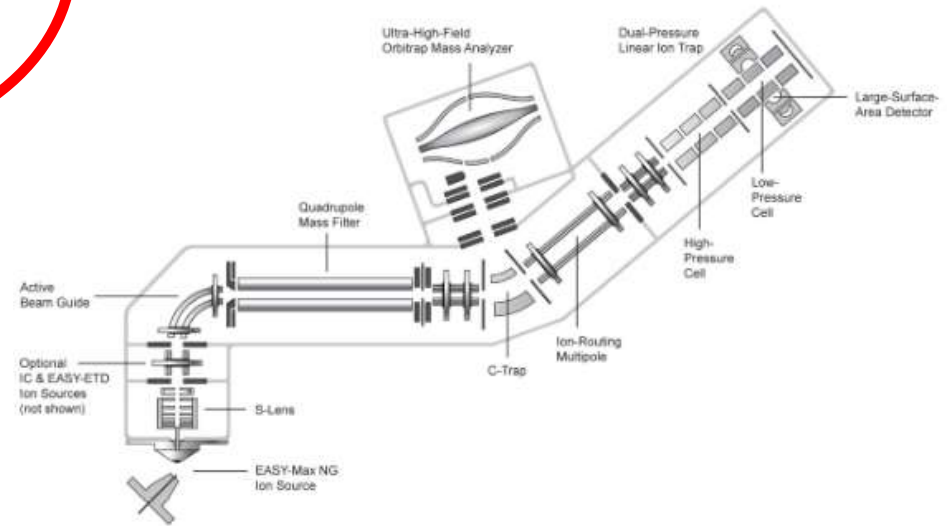
(Ting, et all 2011)

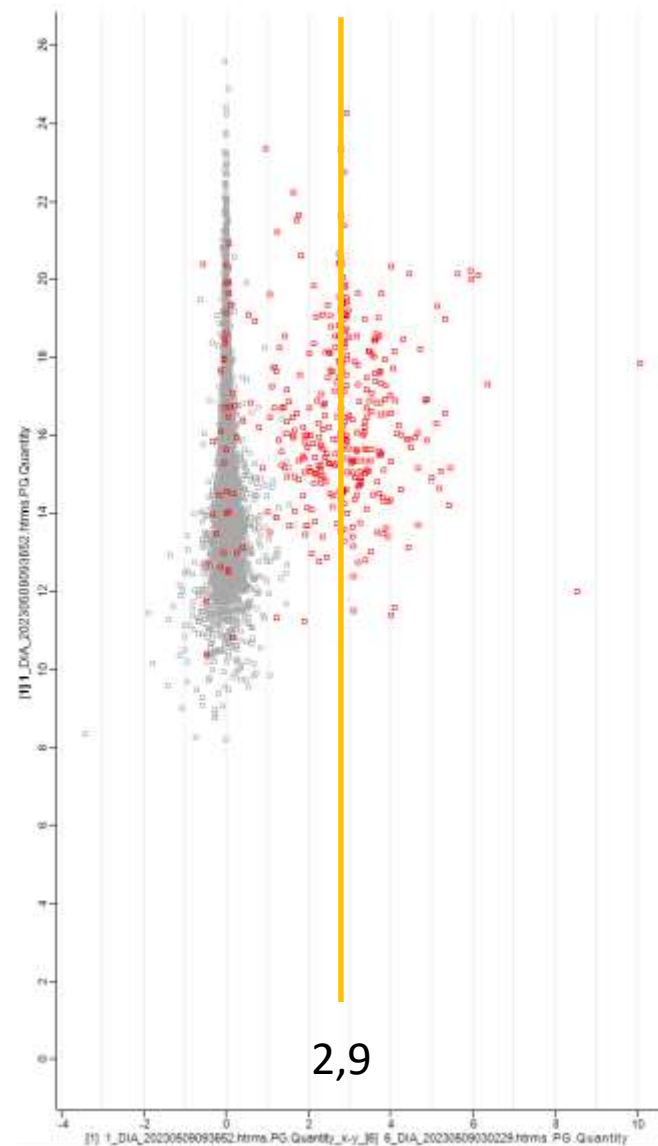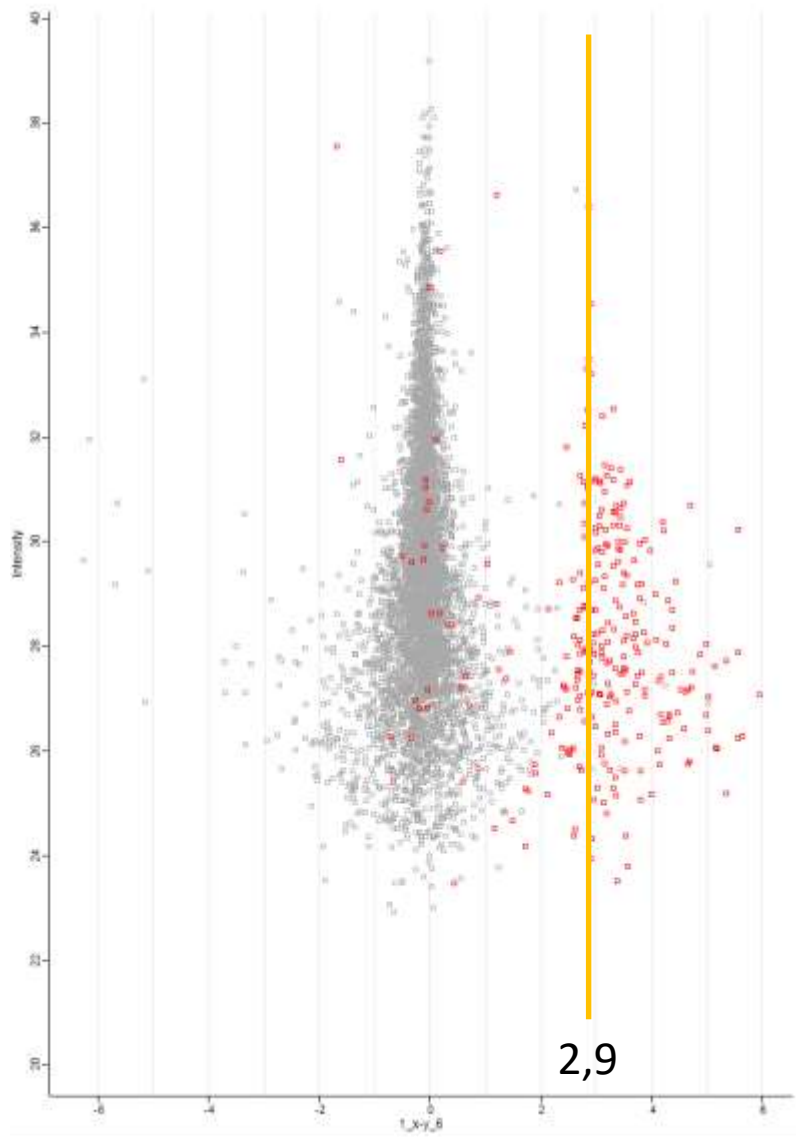# Relative difference four times

MS^2
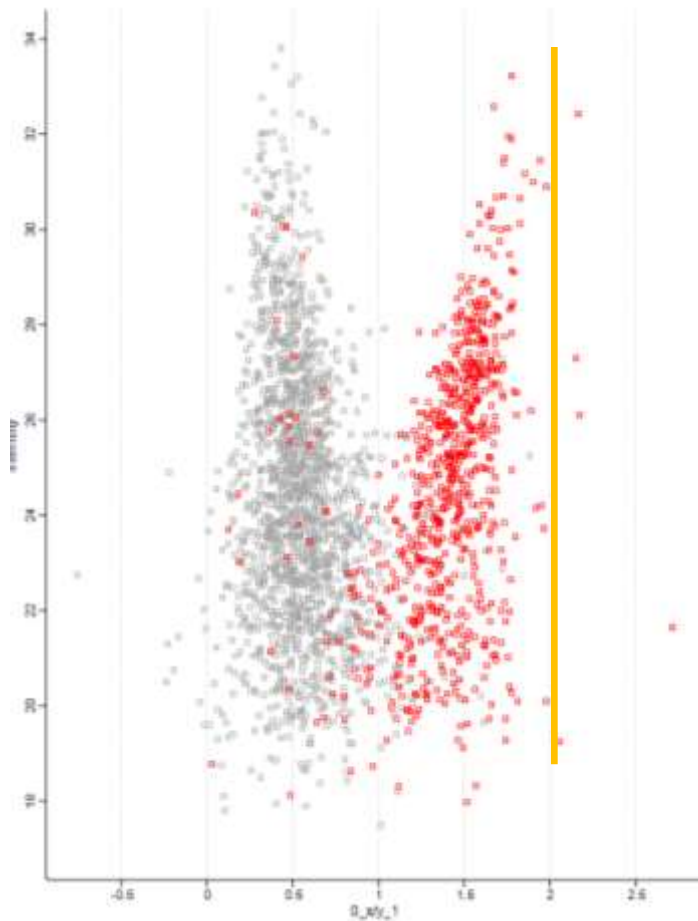
Labell free

MS^3

DDA – 36 hours of instrument time
5758 IDs/ 492 Yeast proteins

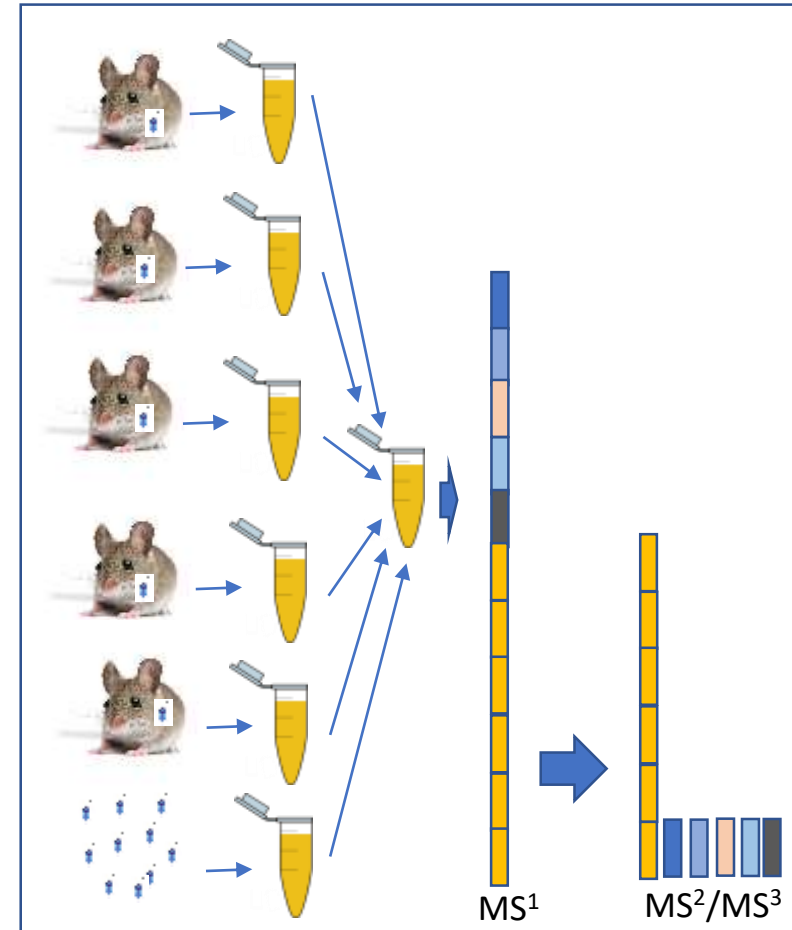DIA – 20 hours of instrument time
7165 IDs / 721 Yeast proteins

TMT - MS3 – 18 hours of instrument time
8898 IDs / 800 Yeast proteins

Real Ratio 7,5 – 2,9 in Binary log
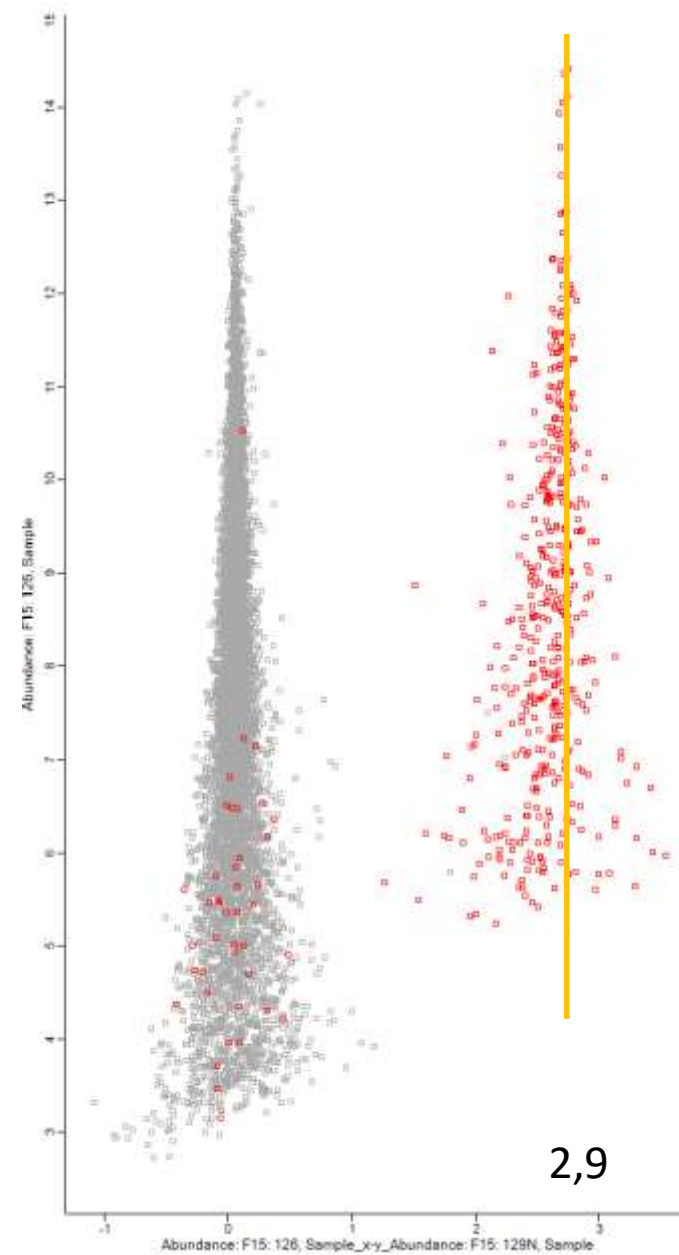
Why use TMT insted of Labellfree ?

- Save instrument time
- One chanell can be carrier
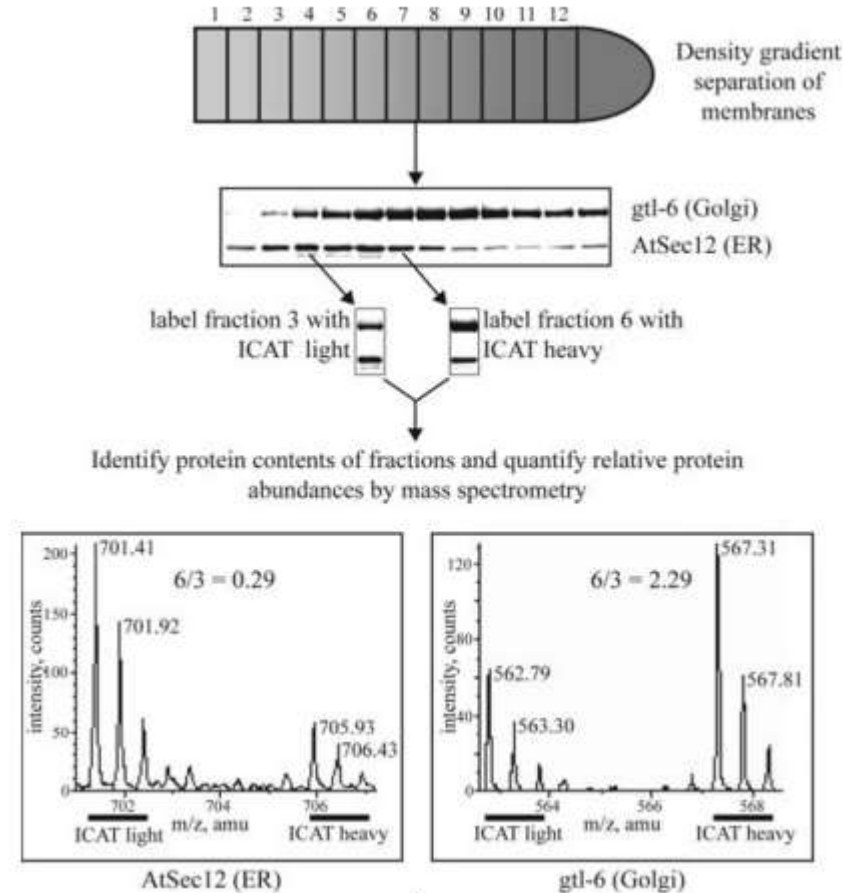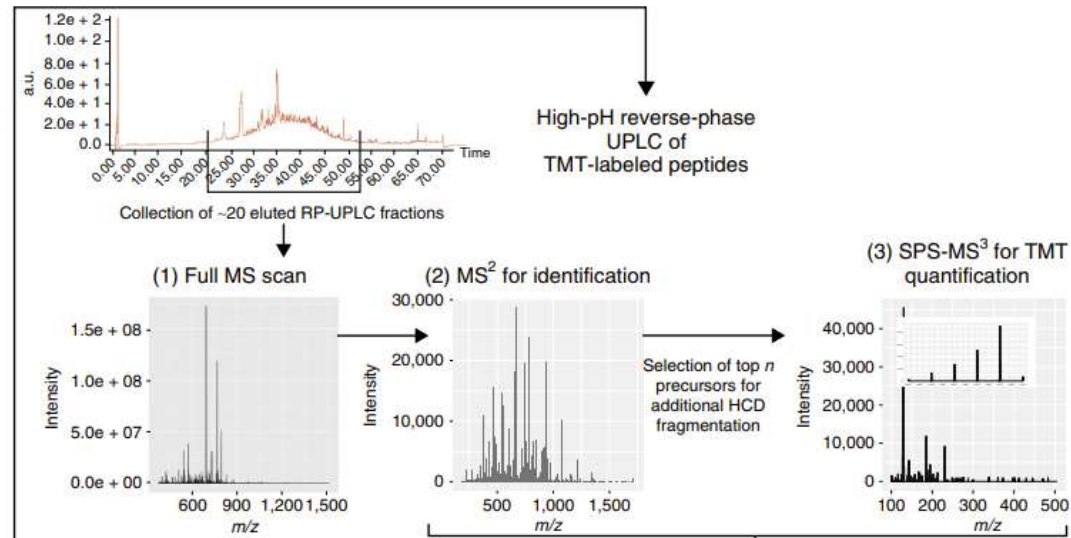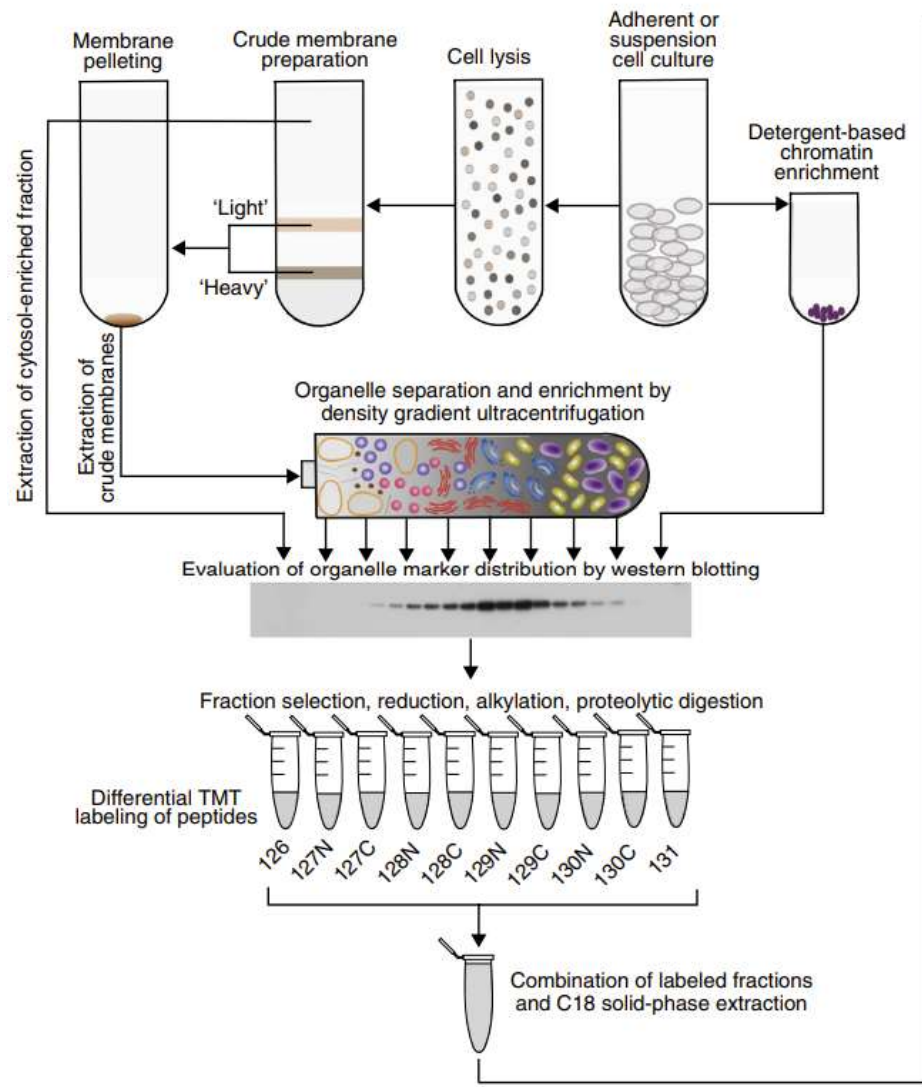- Suberb quality of quantification

Selection of available advanced methods

- Available at our facility as a service

- TMT based intracellular localisation – LOPIT

- TMT based thermal proteome stability – TPP

Localization of Organelle Proteins by Isotope Tagging (LOPIT)

- 2004 K. S. Lilley, MCP
- Developed into several variants
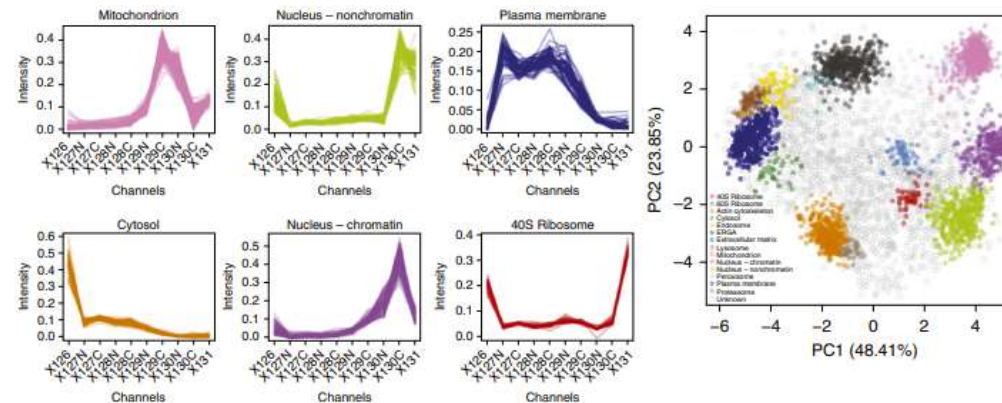- Incorporated isobaric labeling
- Machine-learning software packages

hyperLOPIT - 2017 , K. S. Lilley, NatureProtocols

LOPIT, hyperLopit
LOPIT - DC

- Same principle
- Different organelle separations methods
- Well-established workflow
- Complet R analysis toolset **pRoloc**
- https://lgatto.github.io/pRoloc/
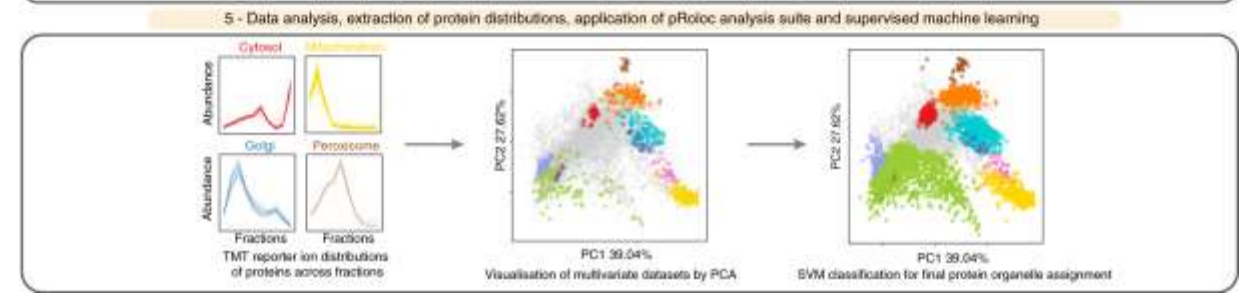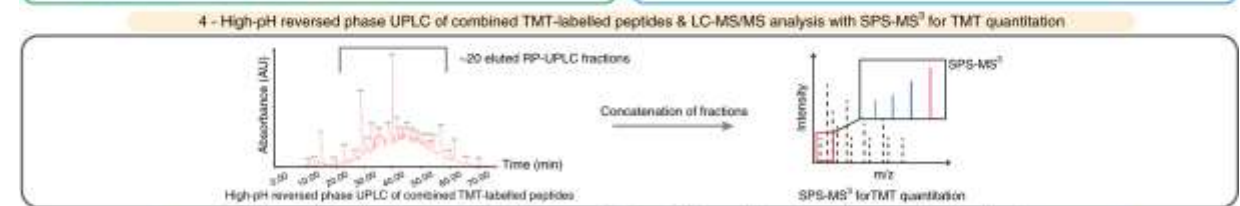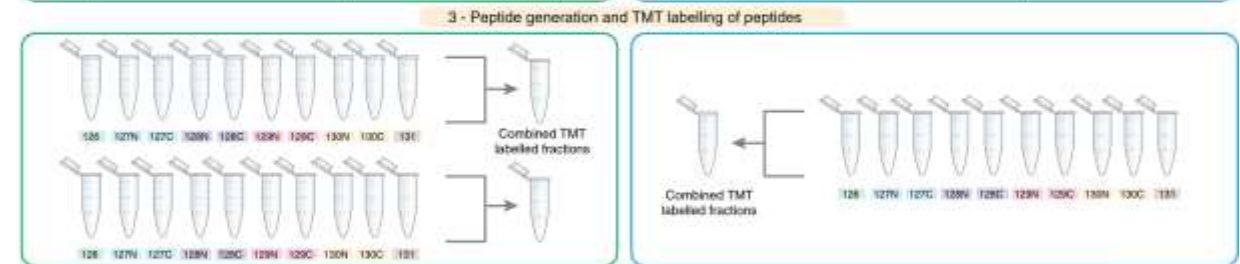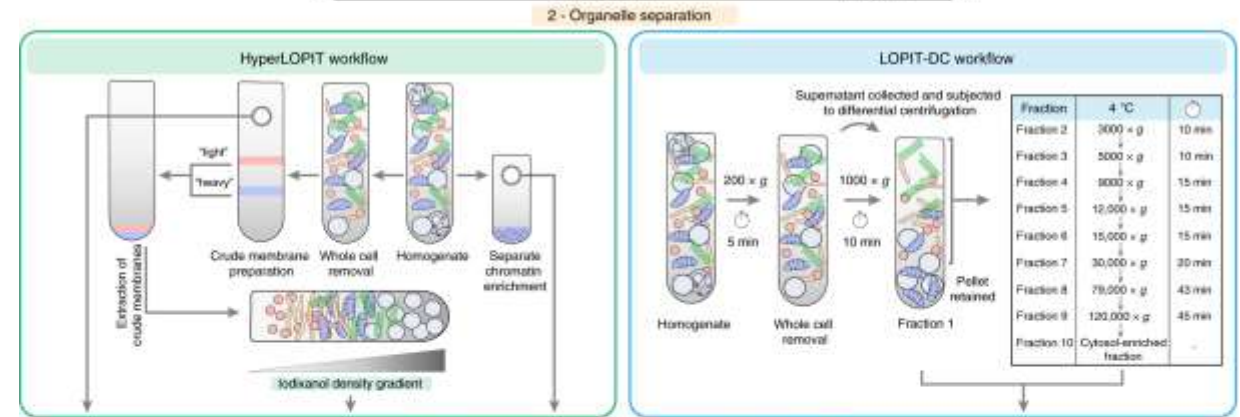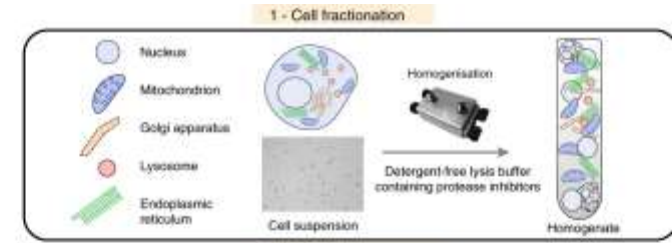
Article

Reduced mitochondria provide an essential function for the cytosolic methionine cycle

Justyna Zítek [1], Zoltán Füssy [1], Sebastian C. Treitli [1], Priscila Peña-Diaz [1], Zuzana Vaitová [1], Daryna Zavadska [1], Karel Harant [2], Vladimír Hampl [1 3]

Justyna Zítek

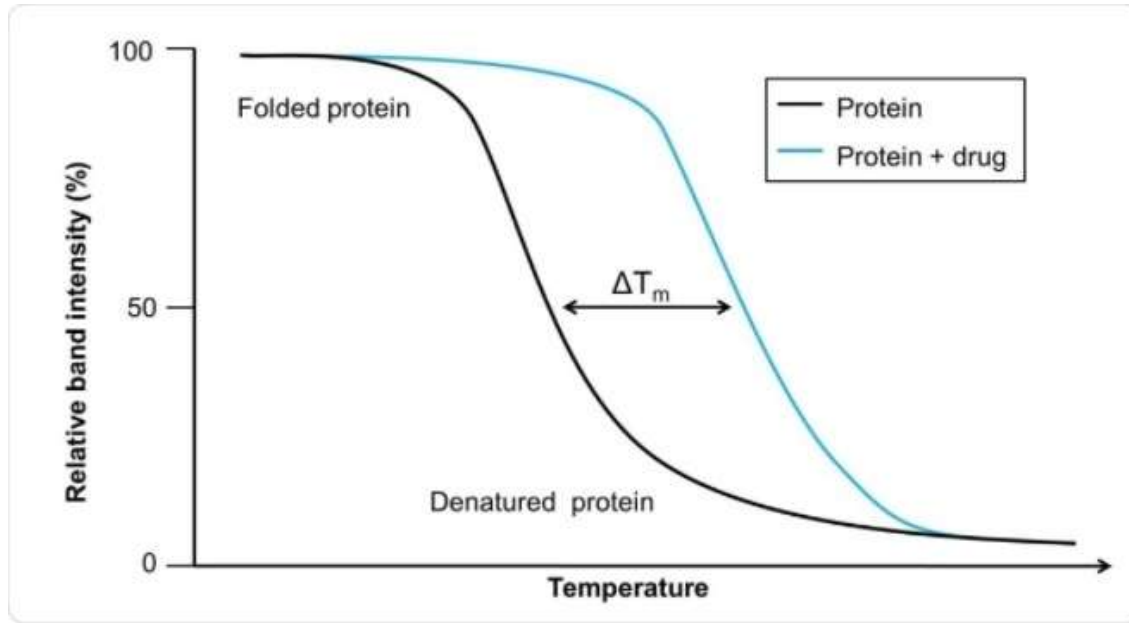# Anaerobic peroxisomes in *Mastigamoeba balamuthi*

PNAS

Vol. 117 | No. 4

Tien Le, Vojtěch Žárský [iD], Eva Nývltová, +5, and Jan Tachezy [iD] ✉ Authors Info & Affiliations

Thermal proteome profiling (TPP)

- Type of Thermal shift assay

- Working with whole live cells - *Cellular thermal shift assay (CETSA)*

- Modification of CETSA – **TPP.**

- Instead of imunochemistry detection use untargeted LC/MS detection. Melting curves detected simultaneusly for thousands of proteins.

Principle of workflow

- Introduced by Dr. Savitski in 2014

- Existing R package for data interpretation - https://github.com/DoroChilds/TPI

- Study of drug interactors

- Design as temperature dependent or dose dependent

- Study of natural molecules target – ATP (2019 Savitski Nature Com.)
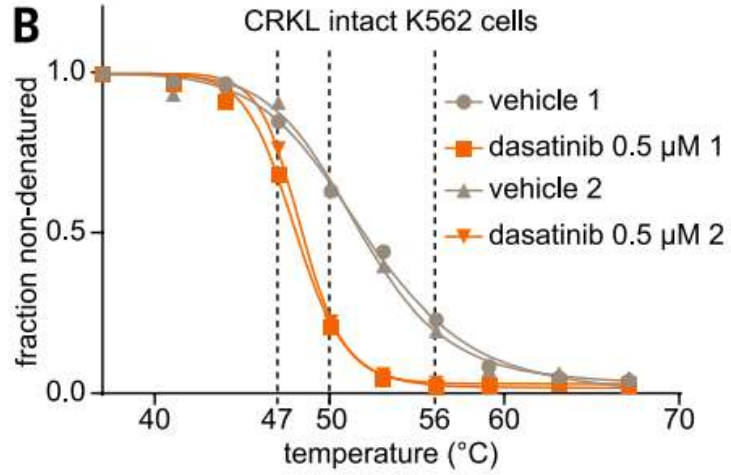
- InVitro/ExVivo samples
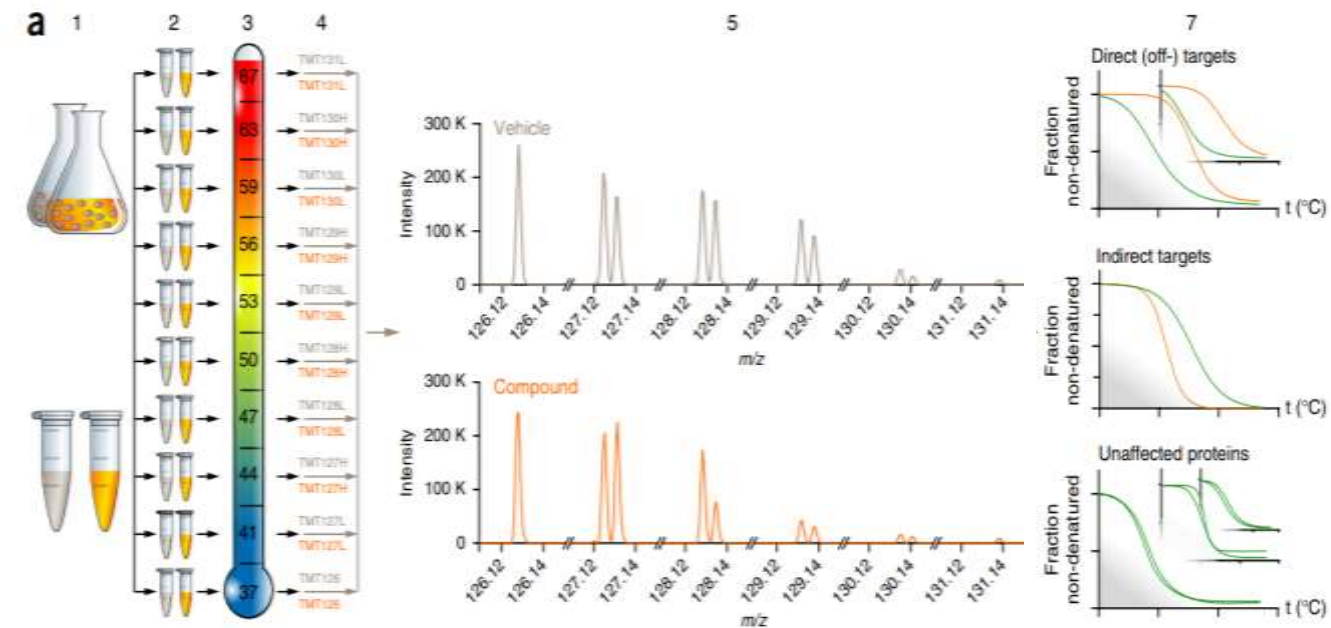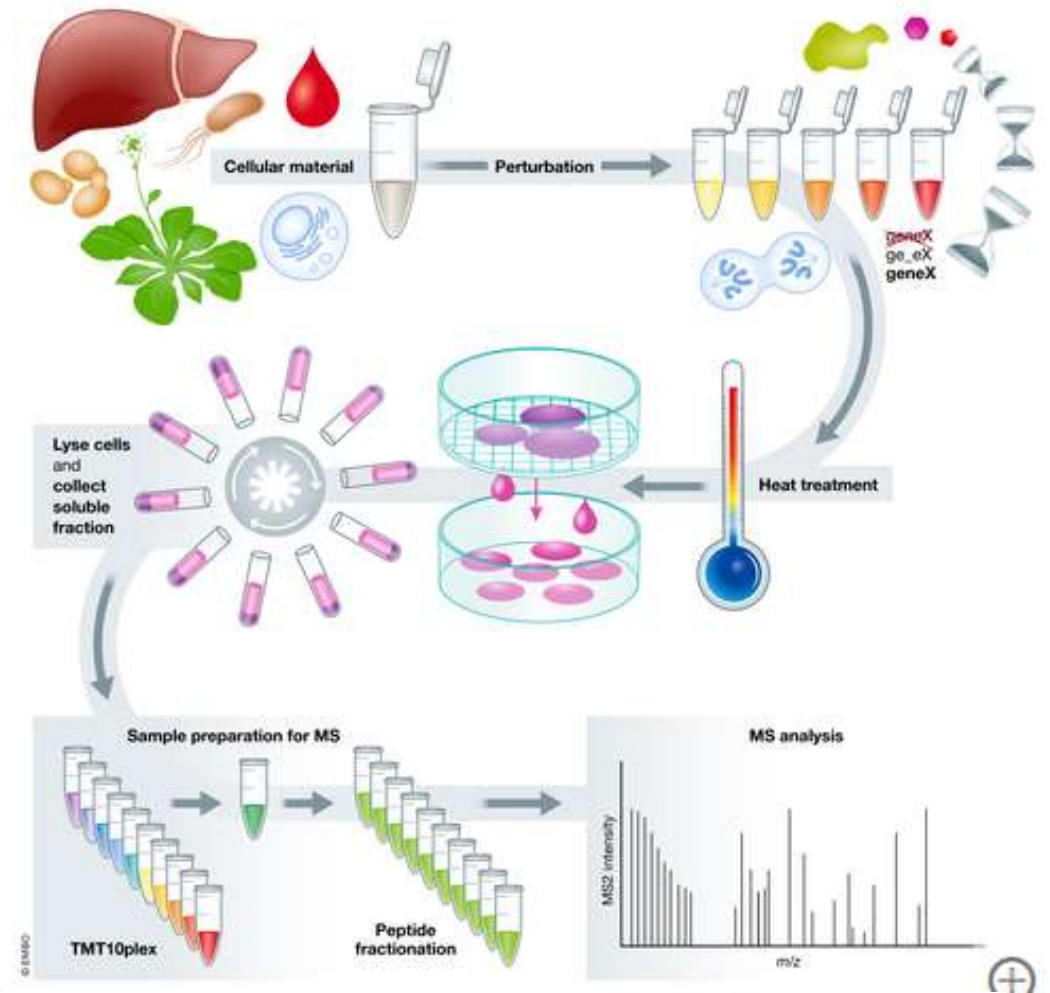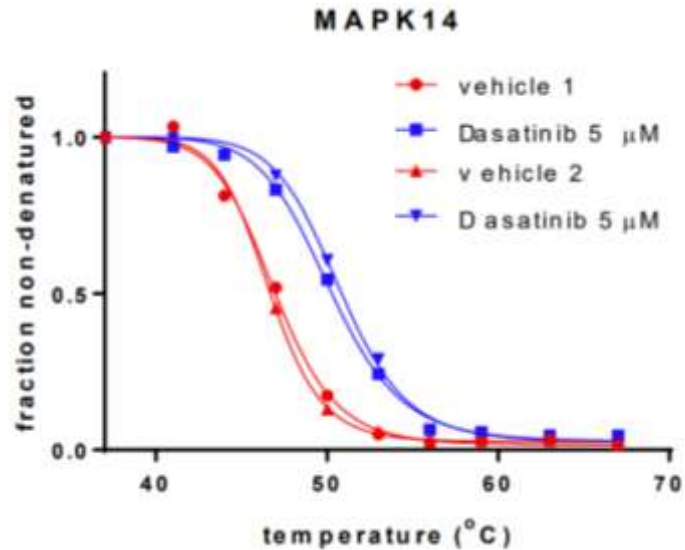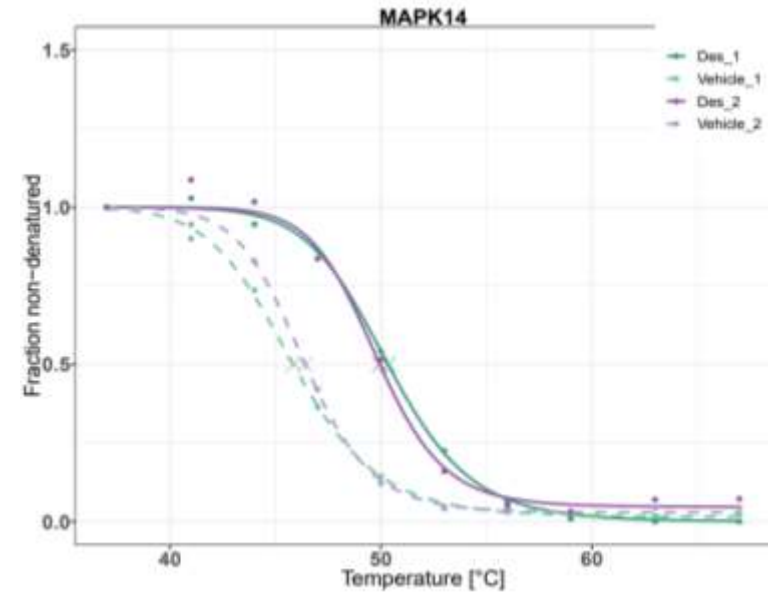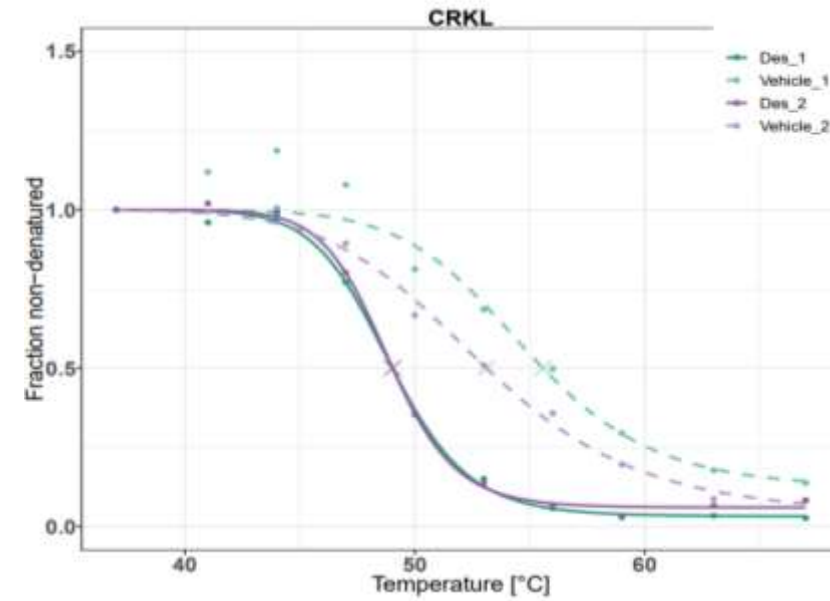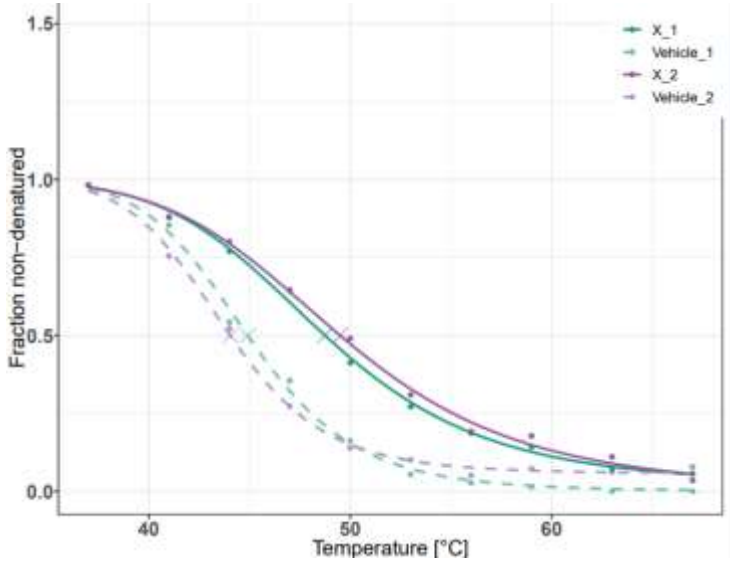

2014, Savitski , Science
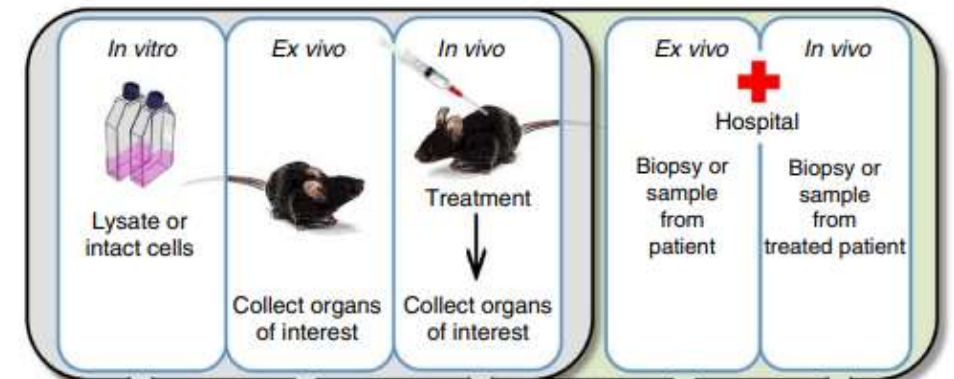
Dasatinib control

Our results
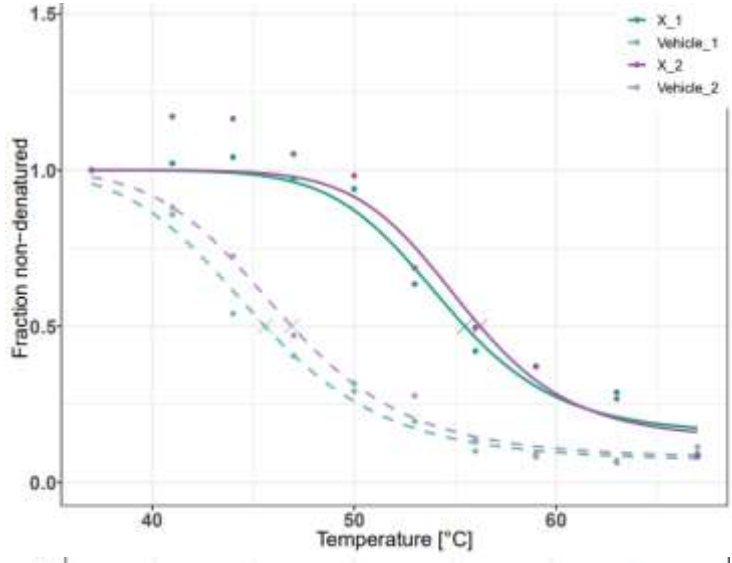

Michal Dibus
Brabek lab

Compound X results - Migrastatic

Original compound
7 candidates

Compound X
20 candidates

Known target
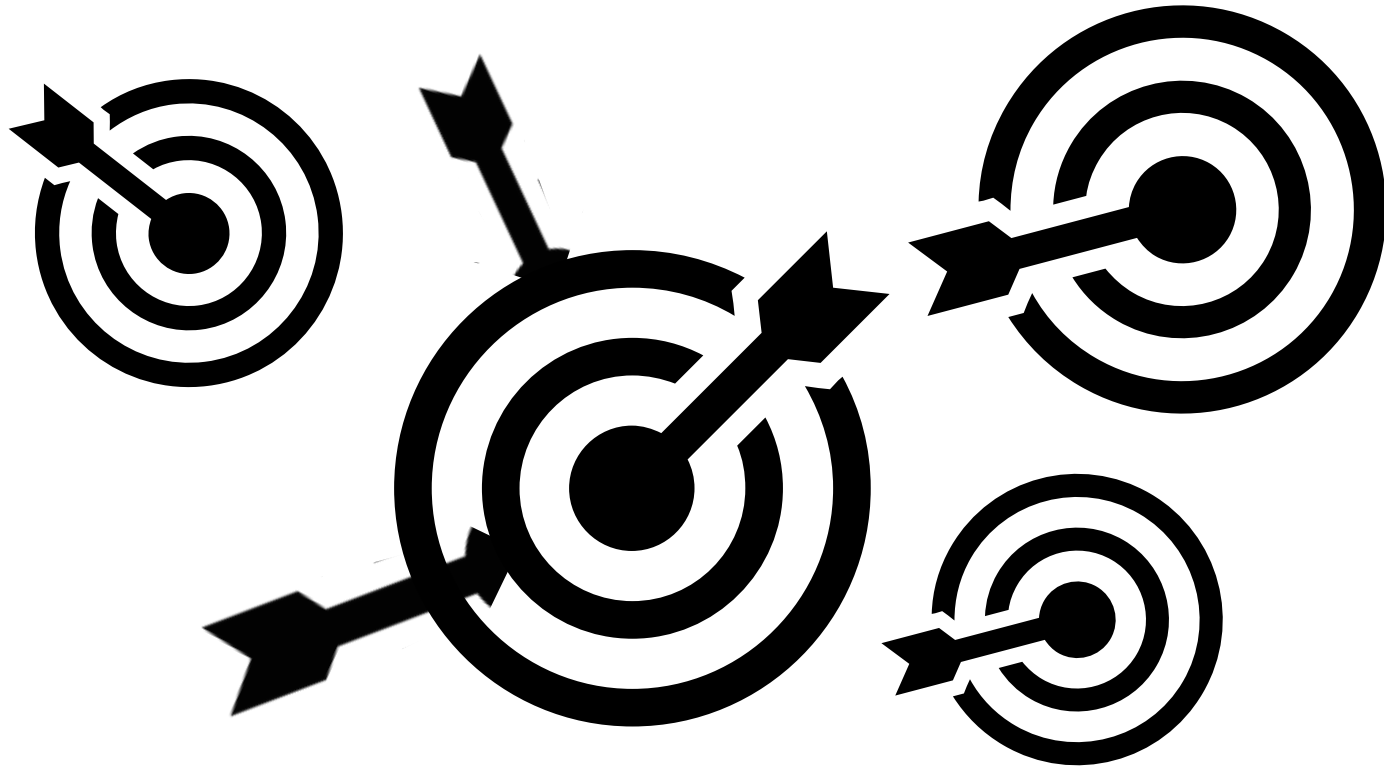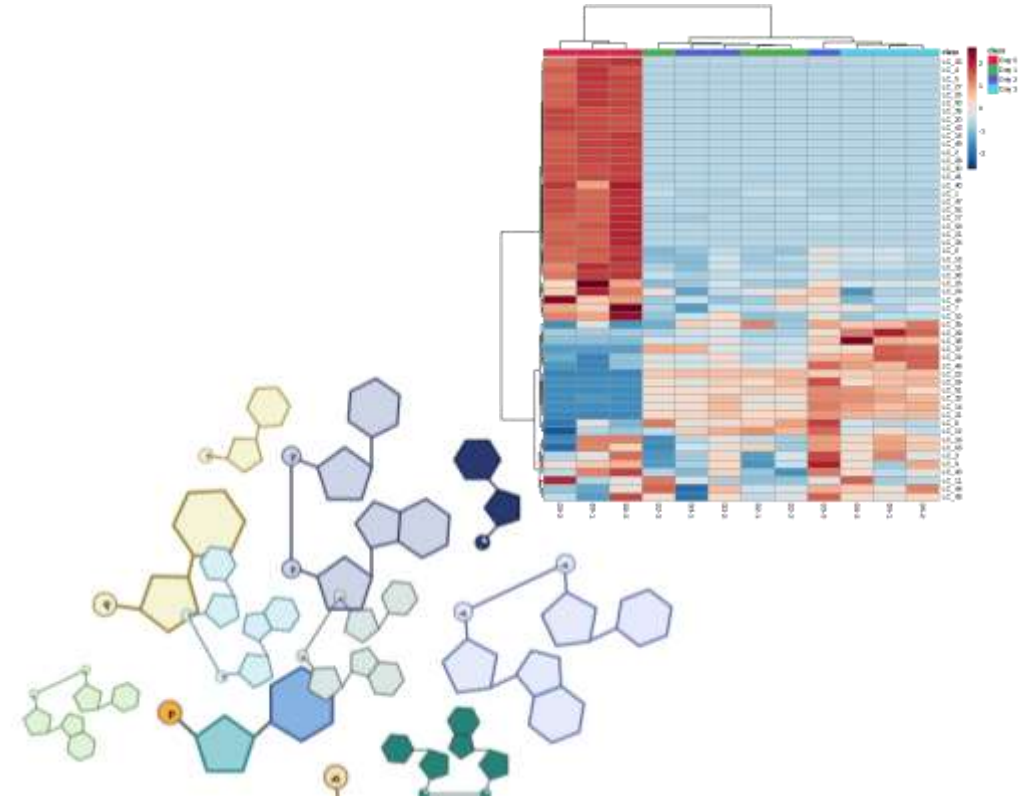
Potential new target
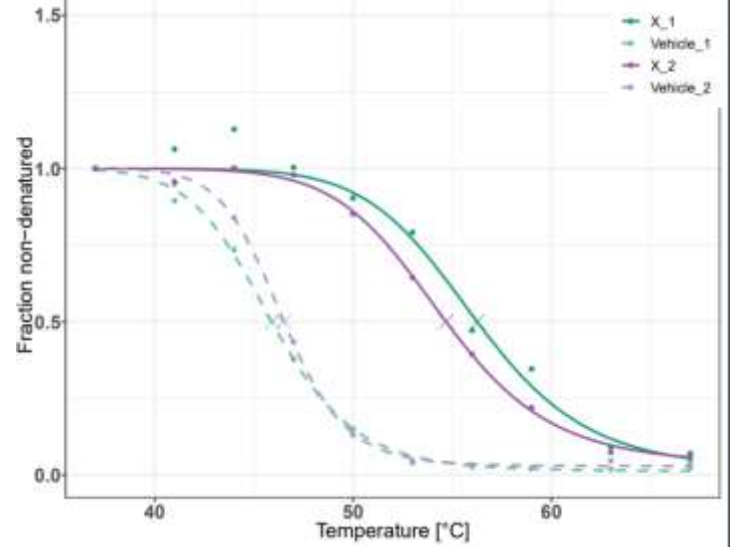
UNTARGETED METABOLOMICS

UNTARGETED METABOLOMICS

- metabolite profiles of control and test samples

- **advantages** – covering a large number of compounds, explain given phenotype of test samples

- **challenges** – many diverse metabolites, data analysis

app.iorender.com
Bathe O.F. et al., 2014
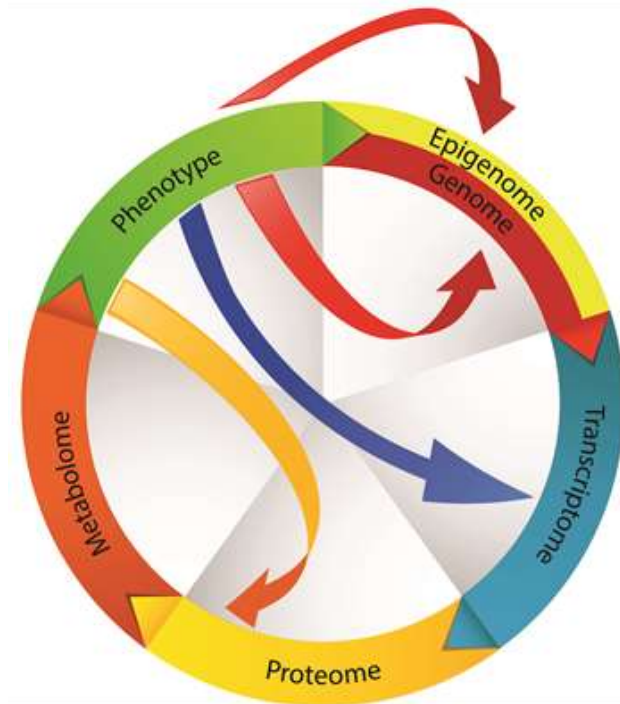
IDEAL WORLD of the measurement

PHENOTYPE

EXPOSOME

Complexity

Metabolome

Genome

Transcriptome

Proteome

app.biorender.com

INSPIRED: Ivanisevic J. et al., 2018

IDEAL WORLD of the measurement

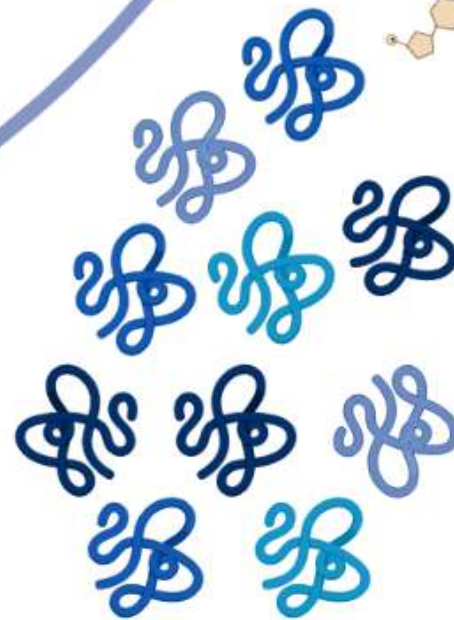**PHENOTYPE**

METABOLOMICS

EXPOSOME

PROTEOMICS

GENOMICS   **TRANSCRIPTOMICS**

Complexity

Metabolome

hmdb The Human Metabolome Database

Genome   Transcriptome   Proteome

app.biorender.com

INSPIRED: Ivanisevic J. et al., 2018

Ivanisevic J. (2018): Metabolomics as a Tool to Understand Pathophysiological Processes

UNTARGETED METABOLOMICS

GCxGC-MS and LC-MS

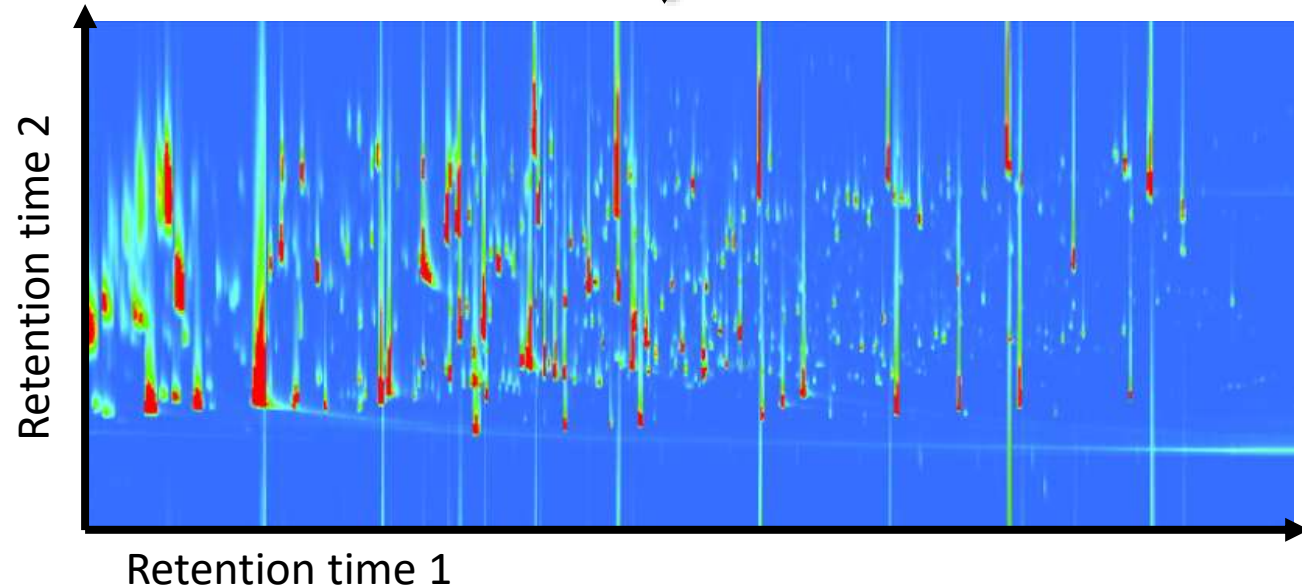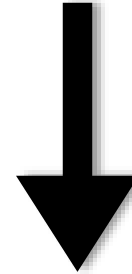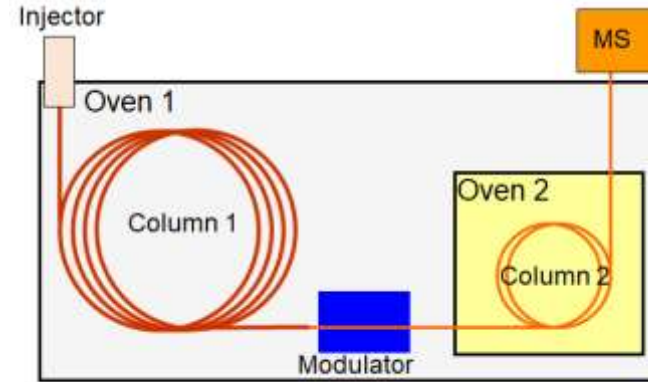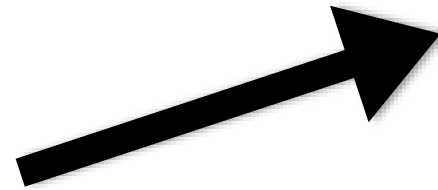| PURINES, PYRIMIDINES | PENTOSE-PHOSPHATE PATH. | FATTY ACIDS, ALCOHOLS... |
AMINO ACIDS	CARNITINES	HYDROCARBONS
COFACTORS	STEROID HORMONES	VOLATILES
GLYCOLYSIS, TCA		

Complementary Techniques

GCxGC-MS
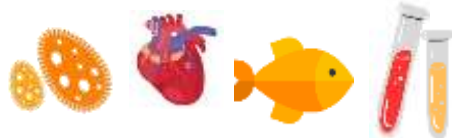
- 2D comprehensive technique
- two separation mechanisms

Pegasus® 4D GCxGC-TOF/MS *(Leco Corp.)*
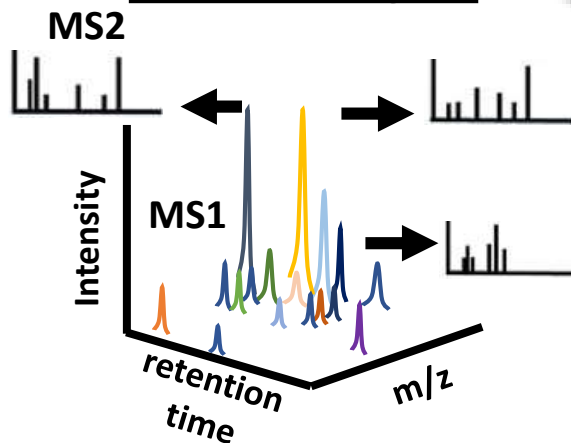
Sample collection/preparation

Data Interpretation

Data acquisition

UNTARGETED
METABOLOMICS
LC-MS

Data analysis

MS2

MS1

Intensity

retention
time

m/z

Compound Identification

SAMPLE

2

1

0

-1

-2

Intensity (counts) (10^6)

59.01329
71.01327
71.01388
73.02953
78.95905

78.95840

96.96889

96.96962

98.91400
101.02371

131.15811

138.97949
138.98018

150.98019
150.97954
168.99077

168.99022
199.00131

173.48145
199.00047

257.23825

fragments

REFERENCE LIBRARY

50   100   150   200   250

m/z
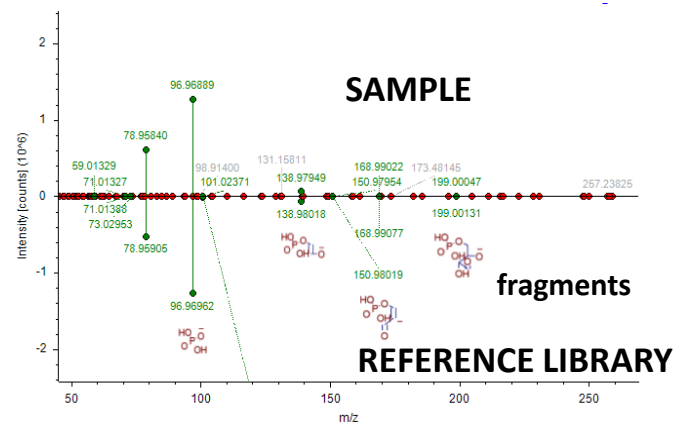
LC-MS UNTARGETED METABOLOMICS

What we need (to know):

• your hypothesis and goals

• nature of your sample

• samples for first trials and replicates for measurement

LC-MS UNTARGETED METABOLOMICS

Profiling

- finding metabolites with statistically significant variations

Compound identification

- determination of the chemical structure of the discovered metabolites
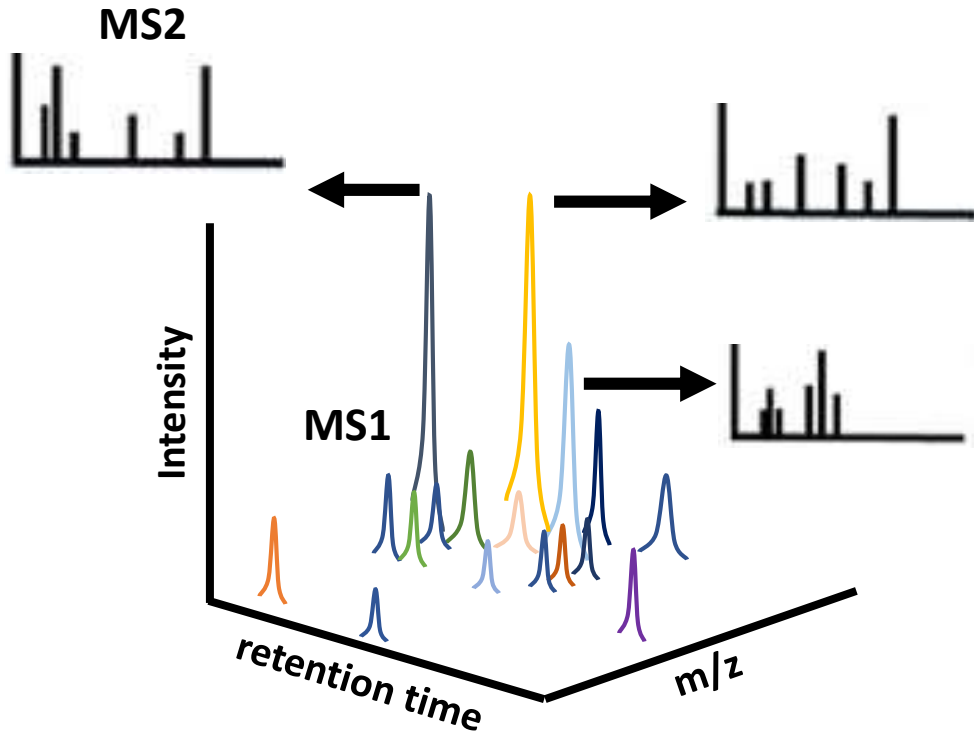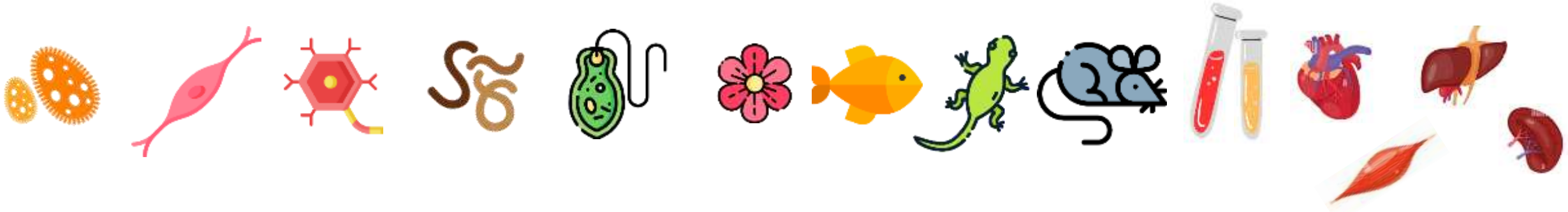
Data interpretation

- uncovering biological connections between the metabolites

LC-MS UNTARGETED METABOLOMICS

• **Profiling**

  • separation and detection must have high reproducibility

  • prepare method to cover broad spectrum of metabolites

  • remove background noise

  • apply methods for efficient metabolites fragmentation

LC-MS UNTARGETED METABOLOMICS

MS2

MS1

Intensity

retention time

m/z

LC-MS UNTARGETED METABOLOMICS

- **Compound Identification**   **Compound Discoverer 3.3**

Glucose-6-phosphate

exact mass 260.029

$C_6H_{13}O_9P$

259.02136
negative mode

LC-MS UNTARGETED METABOLOMICS

• Compound Identification

Glucose-6-phosphate

exact mass 260.029

$C_6H_{13}O_9P$

259.02136
negative mode
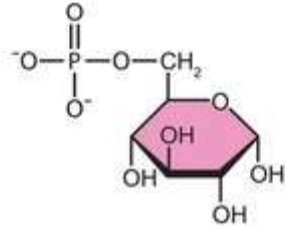
SAMPLE

ChemSpider
Search and Share Chemistry

fragments
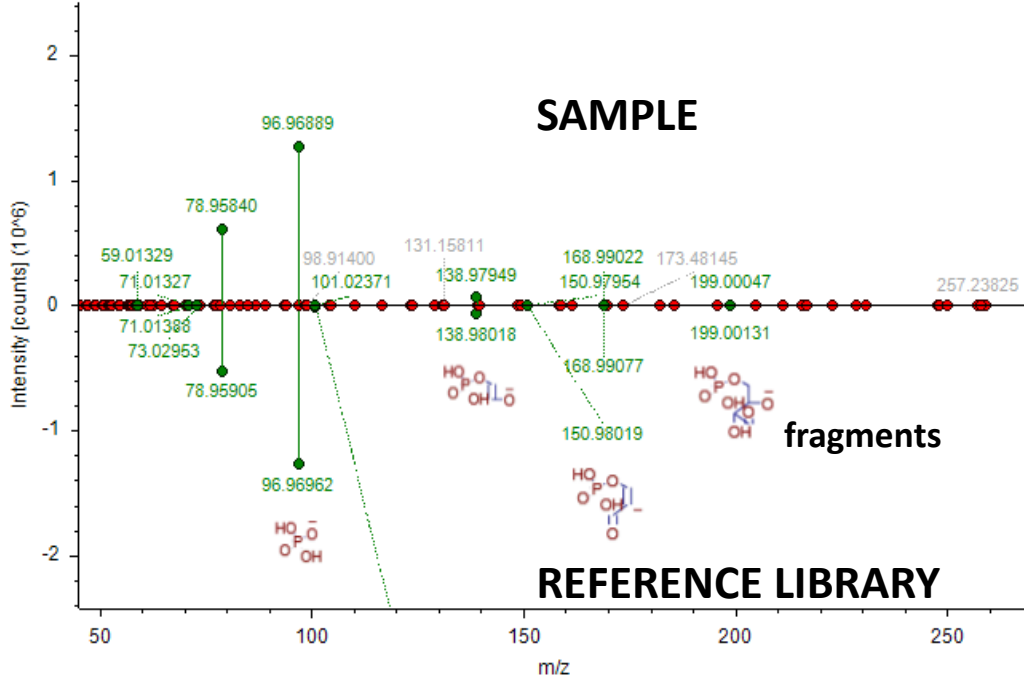
REFERENCE LIBRARY

m/z CLOUD™

LC-MS UNTARGETED METABOLOMICS

- **Compound Identification**

Glucose-6-phosphate

exact mass 260.029

$C_6H_{13}O_9P$

SAMPLE

REFERENCE LIBRARY

fragments

ChemSpider
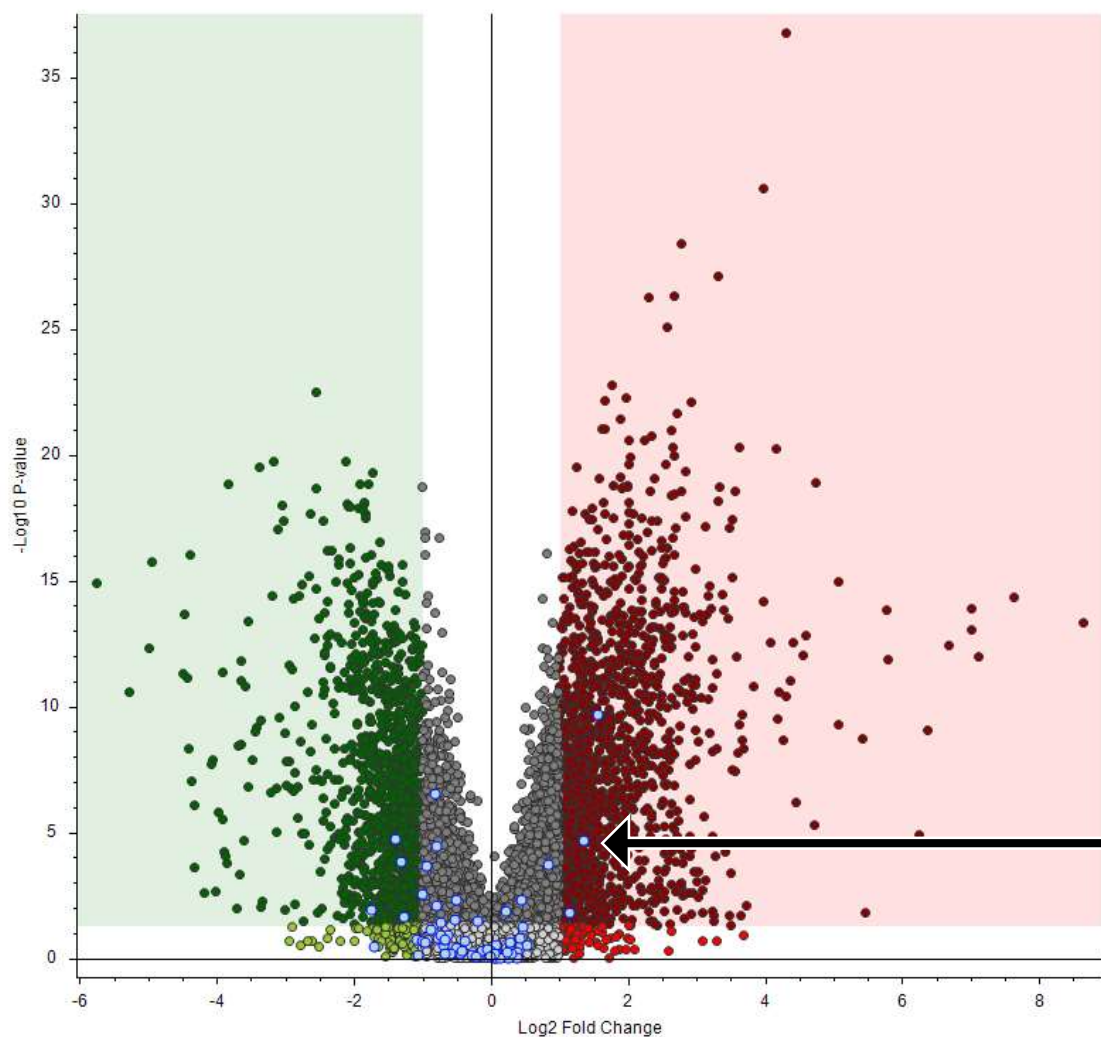Search and Share Chemistry

m/z CLOUD™
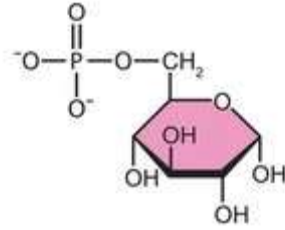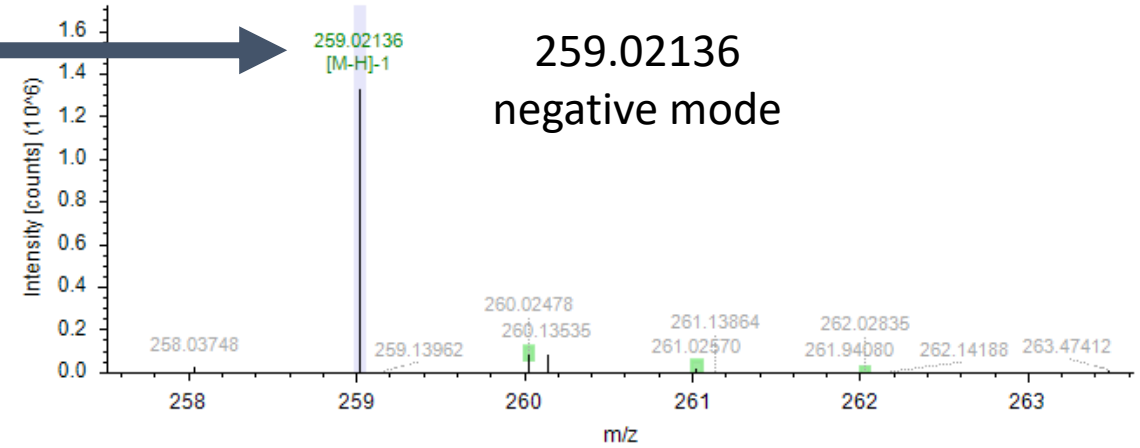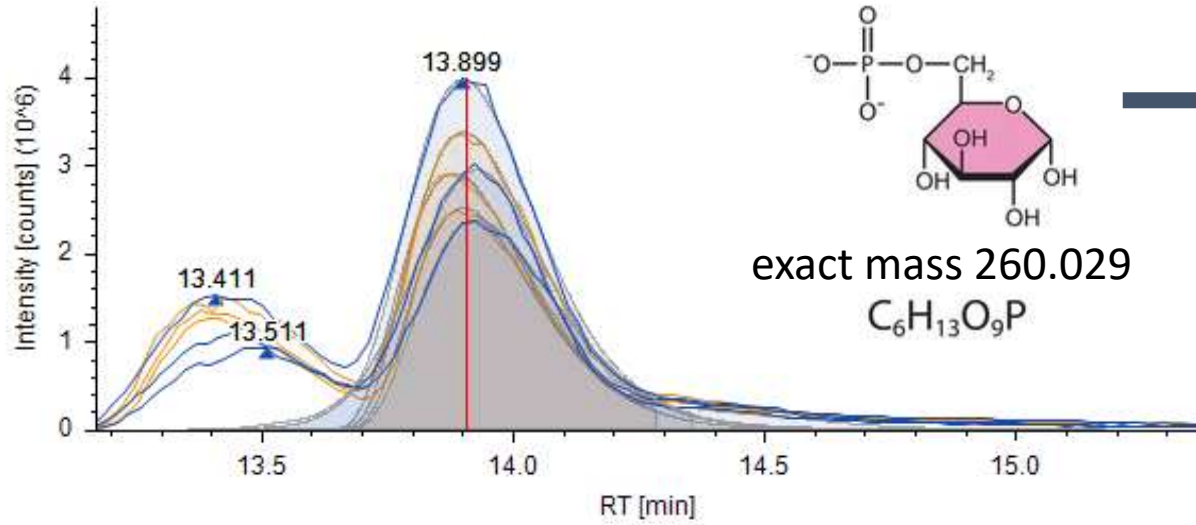
Basic statistical evaluation
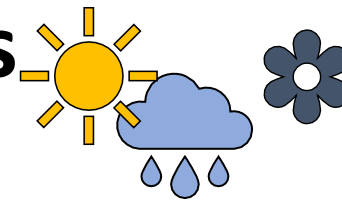
- **Data Interpretation**
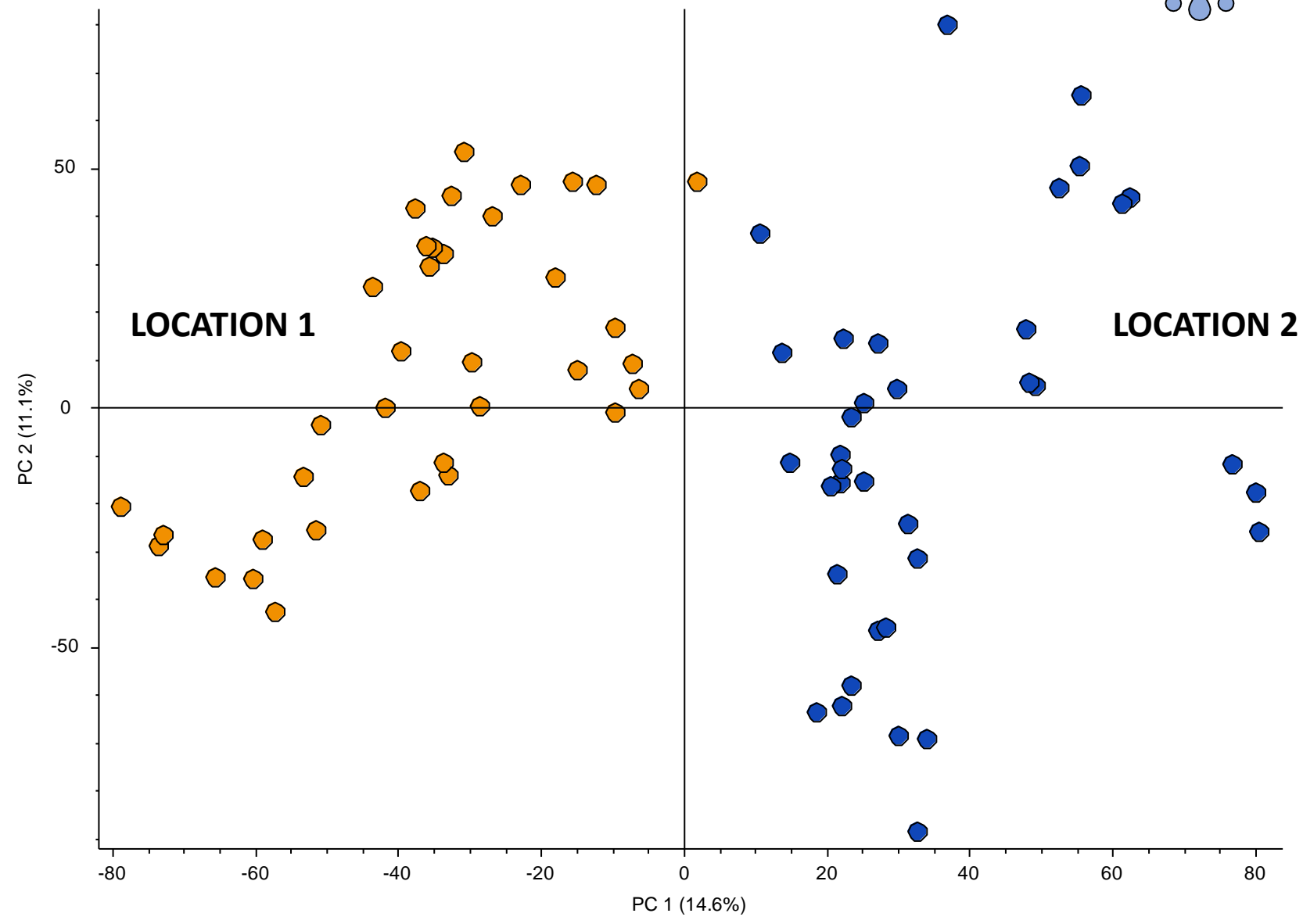
Total metabolite changes between two conditions

🔵 selected interesting compounds

🔴 significantly increased compounds

🟢 significantly decreased compounds

⚪ unchanged compounds

🔵 selected interesting compounds
significantly changed

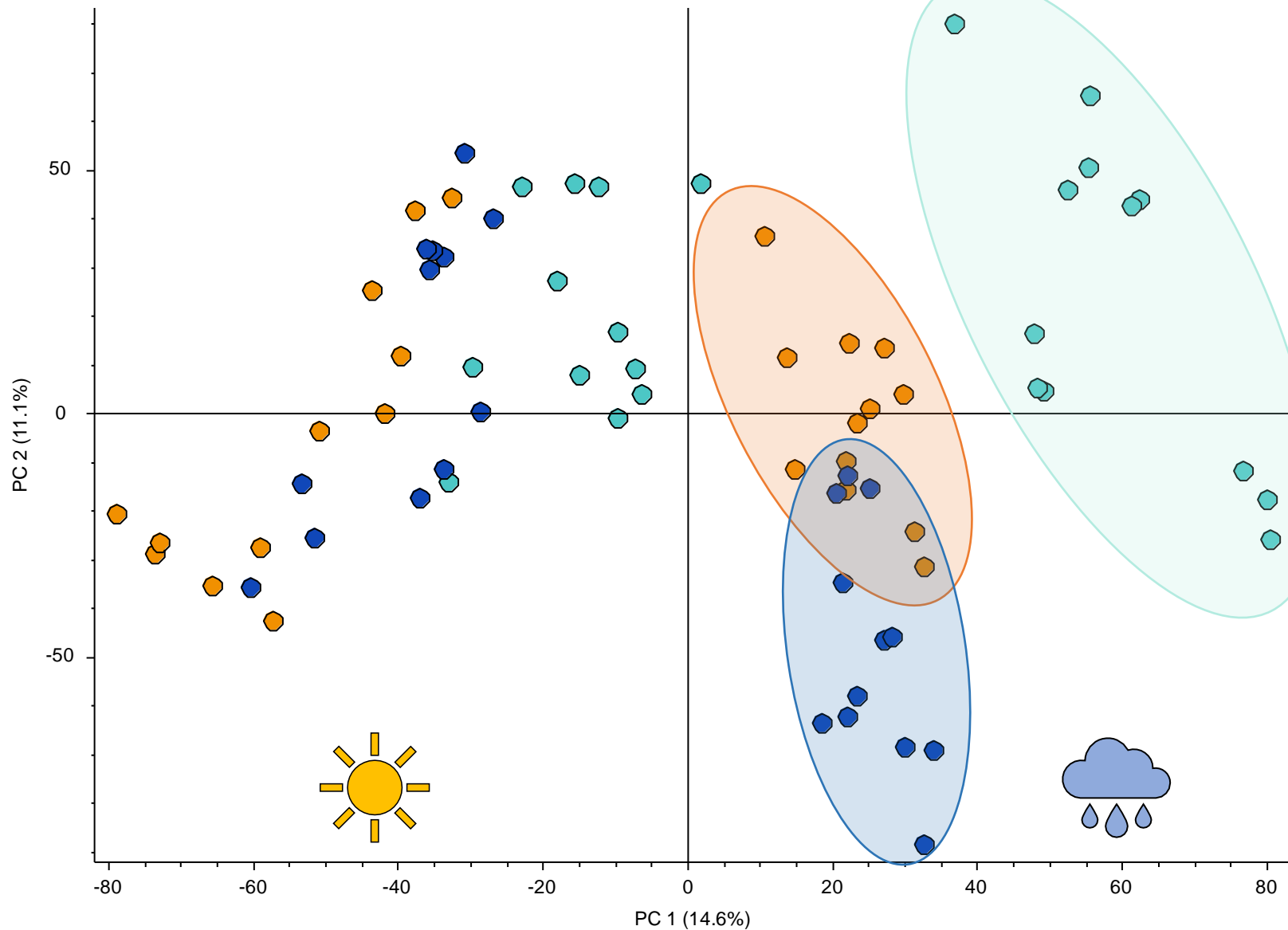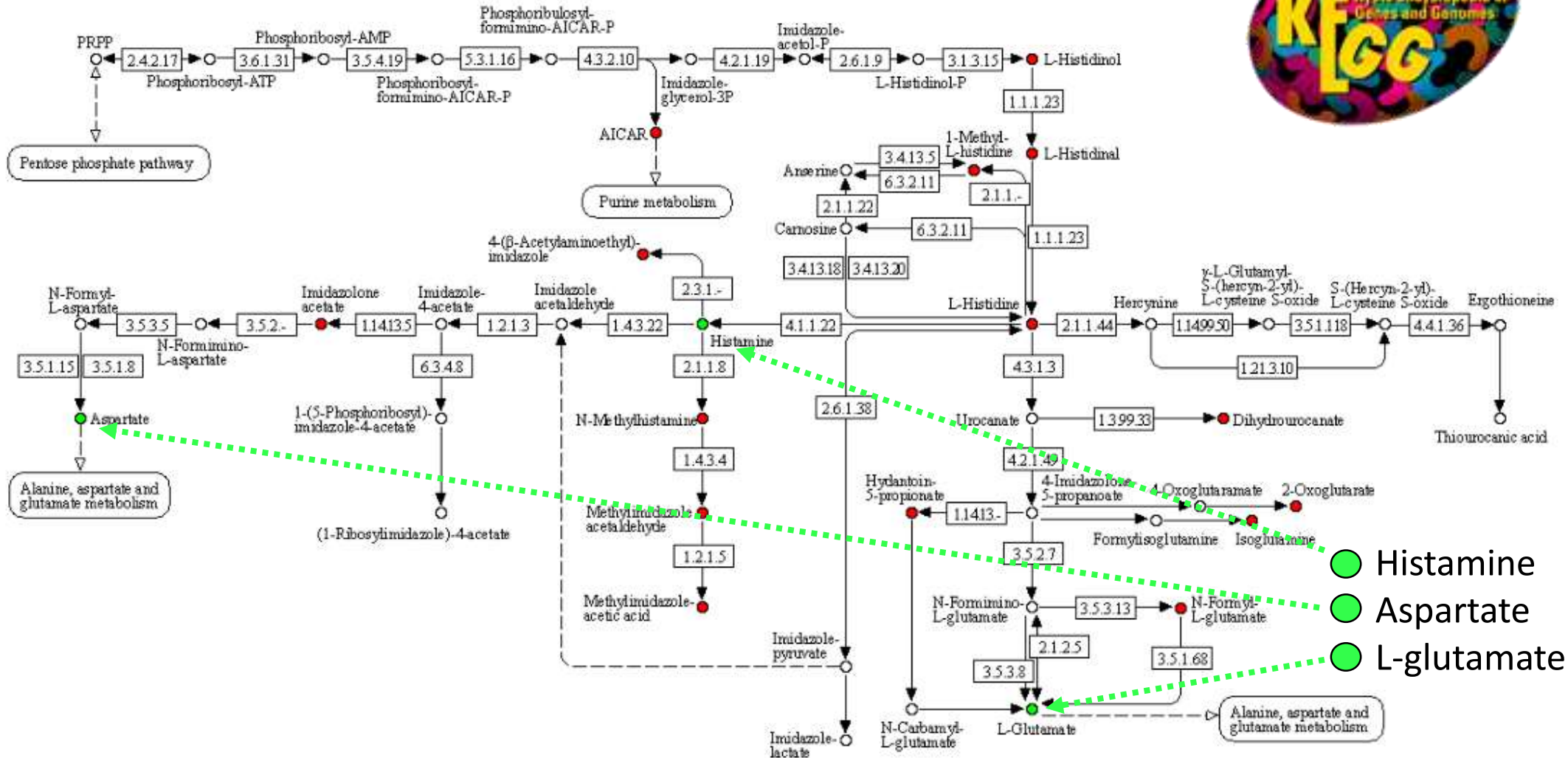# Different locations of the studied samples

Different genotype of the samples

Mapping to metabolic pathways
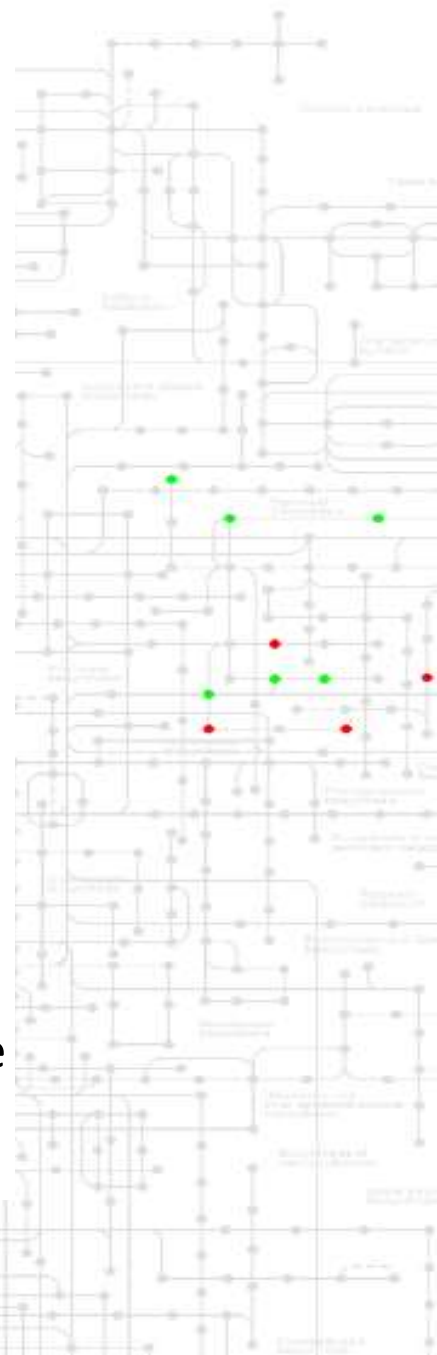
Combination LC-MS and GCxGC-MS
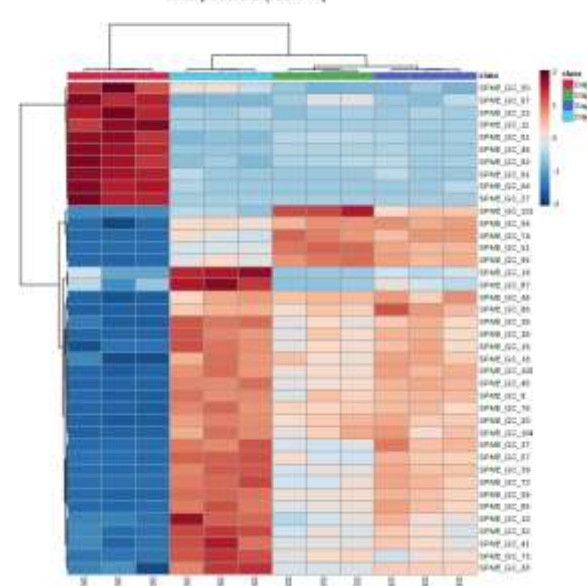
Analysis - **before** and **after** several days of **incubation** with cells
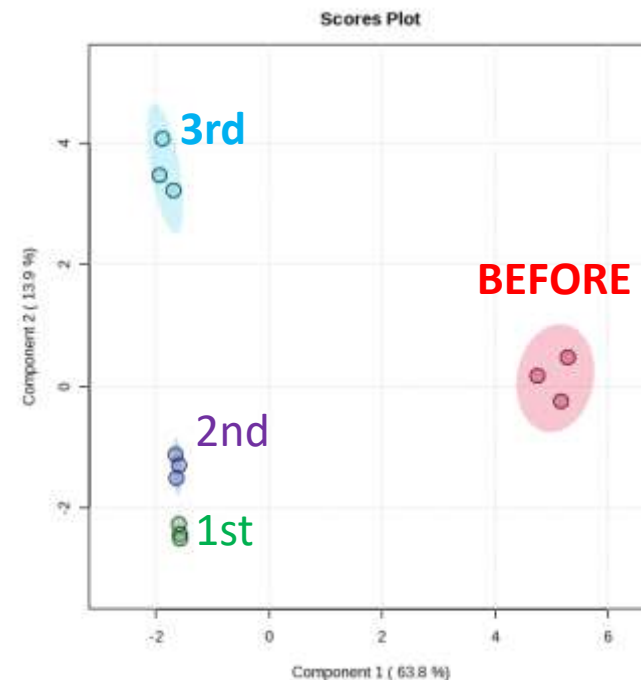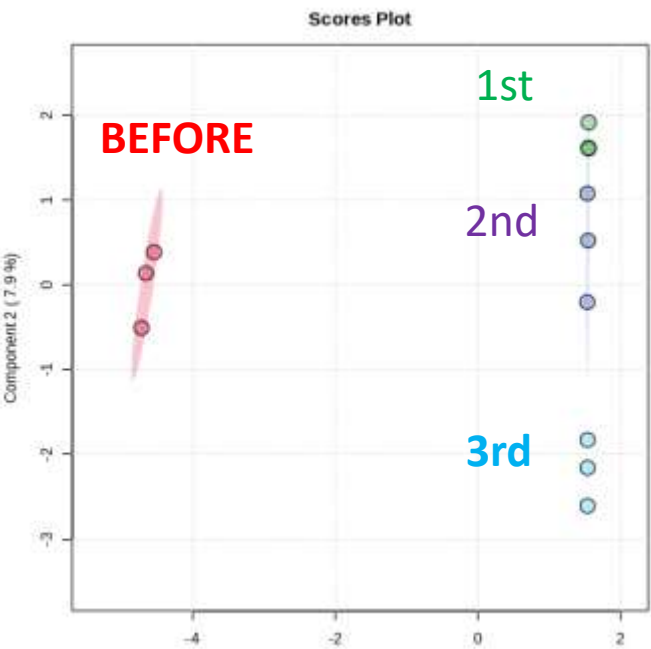
LC-MS

liquid extraction
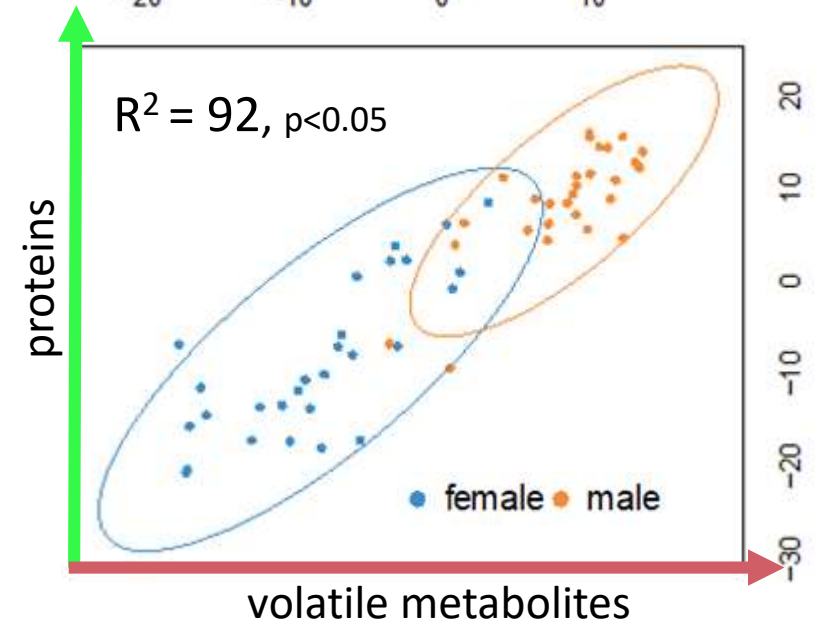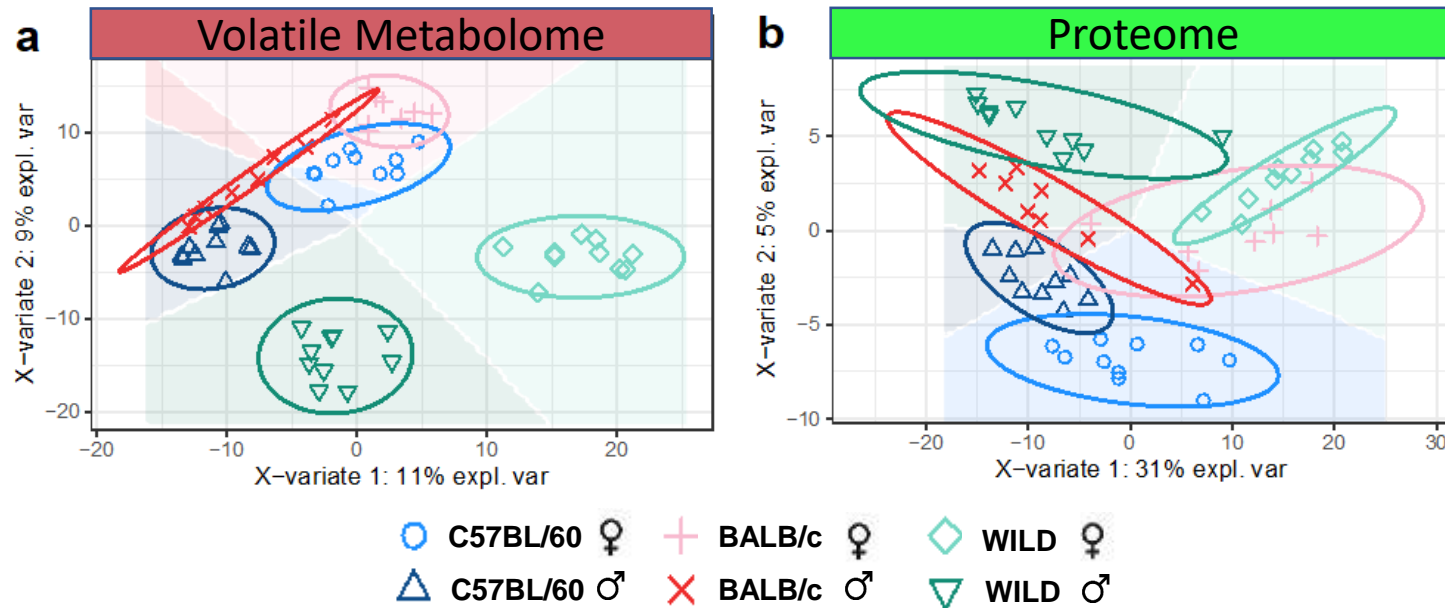AcN:Medium
(4:1)

GCxGC-MS

Headspace SPME
on fiber
(DVB/CARB/PDMS)
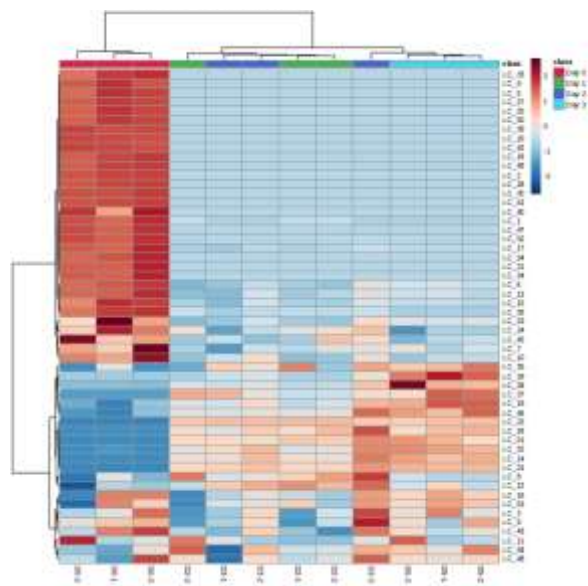
complementarity of the methods

Combination of metabolomics and proteomics

- feromone analysis in mice urine

- differences between strains and sex

BALB/c   C57BL/6   WT

a **Volatile Metabolome**   b **Proteome**

$R^2 = 92$, $p<0.05$

proteins

volatile metabolites

- female - male

○ **C57BL/60** ♀   + **BALB/c** ♀   ◇ **WILD** ♀
△ **C57BL/60** ♂   ✕ **BALB/c** ♂   ▽ **WILD** ♂
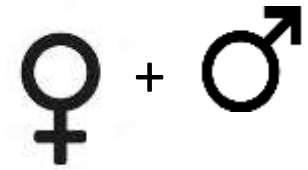
Conclusion

- possible combination of two different methods for UNTARGETED METABOLOMICS

- provided with unsupervised basic statistical analysis

- help with interpretation of data

Future perspective

- isotope labeling experiments
- connecting PROTEOMICS and METABOLOMICS